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THE ANNALS OF APPLIED BIOLOGY

EDITED BY

W. B. BRIERLEY

AND

C. T. GIMINGHAM

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VOLUME XXI FEBRUARY, 1934 No. 1

ZONE LINES IN PLANT TISSUES

II. THE BLACK LINES FORMED BY *ARMILLARIA MELLEA* (VAHL) QUEL.

BY A. H. CAMPBELL, B.SC., PH.D.

(*From the Mycology Department, University of Edinburgh.*)

(With Plates I–III and 4 Text-figures.)

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I. *ARMILLARIA MELLEA* (VAHL) QUEL.

ARMILLARIA MELLEA (Vahl) Quel., one of the commonest and most destructive of parasites, combines an extensive geographical distribution, tropical and temperate, with a degree of polyphagy hardly equalled in the parasitic fungi. This fungus has attracted so much attention from both the systematist and the pathologist that, since the description of *Agaricus melleus* by Vahl (15) in 1777, there has arisen a wealth of literature on the morphology, physiology and pathology of the parasite, which within recent years has been augmented by pathological accounts from North America, Africa, Australia, India and Japan. Numerous figures have been published of the medium-sized toadstool which comprises its sporophore, while its characteristic rhizomorphs are familiar. The rhizomorphs consist of long black strands, rounded or sometimes flattened like bootlaces, which spread throughout the soil and bring about the distribution of the parasite. They are found commonly below the bark of trees killed by the fungus and later radiating in the soil from the dead trees which thus provide bases of attack from which the fungus spreads to neighbouring plants.

Armillaria mellea is remarkable for the variety of plants attacked; for, while numerous coniferous and dicotyledonous trees both tropical and temperate succumb to the fungus, it has also been recorded parasitising such varying hosts as heather (1), potato (17), iris (16) and strawberry (18). Although the fungus is so polyphagous, not all of the apparently susceptible plants with which its rhizomorphs come into contact are infected. To explain this phenomenon the possibility that there are distinct physiological strains has been mooted. Such a case has been described by Childs and Zeller (6) who have shown that, in California, orchard trees, which are usually very susceptible to *Armillaria mellea* on oak-cleared ground, are not infected by the fungus on fir-grubbed land although the crowns of the trees were enmeshed in rhizomorphs, which were giving rise to clusters of sporophores. These trees were examined over a period of years and attempts were made to cause infection by wounding and sawing off roots, but none resulted although the wounds were grown over by the rhizomorphs of the fungus. In consequence, the authors have suggested that there are two physiological strains, one of which naturally infects the oak while the other is found on conifers, chiefly Douglas fir. From the point of view of parasitism of orchard trees, the oak strain is said to be virulent while the fir strain is not: there is, however, no morphological difference discernible in the two strains. This particular problem is at present under investigation, but more evidence will be necessary to establish the existence of these strains as parasitic virulence to orchard trees has already been attributed to *Armillaria mellea* originating from coniferous hosts (12).

The zone lines of *Armillaria mellea* have been found in the wood of the following hosts: *Abies noblis*, *Fagus sylvatica*, *Fraxinus excelsior*, *Larix europea*, *Picea excelsa*, *Pinus sylvestris*, *Pyrus aucuparia*, *Pseudotsuga Douglasii*, *Tilia europea*, *Ulmus montana* and *Veronica propinqua*. These lines, which vary in colour from light brown in the early stages to black in the older rots, were associated in every case with rhizomorphs and in some instances fructifications were present. A number of isolations were made by dissecting out in a sterile manner pieces of the black line and transferring them to malt agar. As the resultant growths were found to agree within the limits of cultural variation, only a single strain isolated from *Veronica propinqua* will be dealt with in detail and may be regarded as typical of the isolations.

The infected *Veronica* from which the isolations were made was a small shrub some 2 ft. in height, which was dying off in a characteristic manner from the centre as a result of all the main roots being attacked

(Plate I, fig. 1). The plant, however, was still alive at the margins where, by means of adventitious roots, it had been able to establish a food supply. Rhizomorphs of *Armillaria mellea* were plentiful in the soil around the bush and could be traced entering the roots where the black lines were numerous in the bark and wood (Plate I, fig. 5). It is believed that the original host of the *Armillaria mellea* was oak.

In the attacked roots the black lines were continuous in both wood and bark as there was no apparent xylostroma occurring in the position of the cambium. Areas of white mycelium which might be regarded as the xylostroma were to be found, however, in the outer layers of the bark. A transverse section across the root revealed a number of black circles some of which included both wood and bark tissues. Successive sections showed that the black circles were sections across elongated oval bodies which had the black line for their circumference. A number of these bodies of various sizes and shapes but always with their long axes parallel to the vertical axis of the root were found embedded in the tissues. The wood itself was white in colour, and had obviously been considerably delignified. In the advanced stages of the decay the tissues were so soft and wet that water could be literally wrung out of such infected roots. The black lines, however, remained intact and were rendered even more distinct by the rotting of the surrounding wood which caused the lines to stand out in relief.

II. DEVELOPMENT OF THE FUNGUS IN CULTURE.

When a piece of wood infected with *Armillaria mellea* is inoculated on to an artificial medium such as oat agar, the hyphae grow out from the wood and enter the medium. Within a short time a tuft of mycelium is formed over the wood inoculum. This mycelial mass is at first white, but later becomes brown in colour. When the tuft is white the mycelium is composed of hyaline, densely protoplasmic hyphae which vary in diameter from 2 to 3·5μ, have few septa and are seldom branched. After a time the hyaline hyphae become darker in colour with more numerous septa, having now a diameter of about 3μ. Branches then begin to be formed close to the well-marked septa and give rise to a chain of bladder cells dark brown in colour, which vary in diameter from 6 to 14μ. These bladder cells are thick walled and their protoplasmic contents have been replaced by a clear fluid. The intertwining of the chains of bladder cells transforms the mycelial mass into the closely knit plate of brown pseudoparenchyma which is so characteristic of the fungus when grown on oat agar. This brown plate or tuft, which Brefeld (3) regarded in his cultures as a sclerotium, then gives rise to rhizomorphs. These are usually developed from the under-surface of the plate, that is the side next to the medium, and frequently all of them arise from a single point. The rhizomorphs thus formed grow down into the medium for some distance and then emerge above the surface of the agar. While submerged in the agar, the rhizomorphs are flattened and white in colour, and are surrounded by a weft of hyaline hyphae which grow out from the rhizomorph into the medium, but immediately the rhizomorph emerges above the surface of the medium it becomes cylindrical and dark brown in colour as the surrounding hyphae disappear and in their place is formed the glossy dark brown rind of the typical rhizomorph. The hyaline hyphae, however, spread along the surface of the medium from the point where the rhizomorph has emerged and form a new mycelial plate which eventually joins up with the original tuft (Plate II, fig. 5). Thus in old cultures on oat agar the entire surface is covered with a brown mycelial plate which may show very marked zonation.

The growth of *Armillaria mellea* in culture varies considerably on different media but, in general, media containing easily available carbohydrates result in the best growth. Such a medium as *C* medium(5) or bread soaked in malt extract solution are very suitable for producing a large amount of vegetative growth and abundant rhizomorphs which may attain a breadth of over 1 in. When this fungus is grown on malt agar the mycelial growth is much scantier and is confined almost entirely to within the medium where it forms a black mass below the surface. The rhizomorphs, too, are black in colour and much more natural in appearance. A curious feature of the cultures is that bodies resembling sclerotia may be formed in the depths of the medium as is seen in Plate II, fig. 6. It can be seen that this body is very similar in structure to a sclerotium having a white medulla and being surrounded by a black rind except where in contact with the glass. From these bodies in the depths of the medium arise the rhizomorphs which penetrate to the surface and grow out free from the medium. Another curious phenomenon in connection with these sclerotium-like bodies is seen in older cultures. Plate II, fig. 2 shows the reverse of two slope cultures of *Armillaria mellea* which have been drying out and in consequence there has been formed a black plate down through the medium to make contact with the glass, thus enclosing the whole of the bottom portion of the slopes within a black rind except, of course, where the slope is in contact with the glass. It can be seen that this black plate, which is composed of bladder hyphae, has greatly diminished the rate of drying of the enclosed portion of the medium and thus seems to suggest the formation of a sclerotium which contains, in part, the matrix upon which it is growing. The mycelium in the cultures on malt agar is generally formed within the medium, but when exposed at the surface it has a black shiny appearance quite different from the cultures on oat agar. The brown hyphae formed in the medium, too, are different from those formed on oat agar, as they are generally stouter, more irregular in shape and frequently branched. Bladder cells are also formed, but they are much smaller than normal, being only on an average about 6μ in diameter, and filled with densely granular protoplasm. There can be no doubt that these hyphae are abnormal forms as a result of their submergence in the depths of the medium. White mycelium is rare in malt-agar cultures and is only seen in the early stages and as the medulla of the sclerotium-like bodies in the medium.

An interesting phenomenon is seen when the tips of rhizomorphs developed in culture are cut off and transferred to a fresh medium. The rhizomorph tip about $\frac{1}{8}$ in. long is laid flat on the medium and immediately, without any apparent connection with the substratum, the tip continues its growth so that at the end of 10 days the rhizomorph has become about an inch long and has given rise to a number of branches. About this time, the connection with the medium becomes evident to the naked eye as a dark halo around the point of inoculation, but it is not very marked and in no way resembles the tuft or mycelial plate in which the rhizomorphs usually have their origin. Thereafter, while the apical growth continues vigorously, more and more branches appear until there is a network of rhizomorphs spreading over the medium. No aerial mycelium other than rhizomorphs can be seen in such a culture on malt agar, but the rhizomorphs may penetrate into the medium for part of their course. This is seen in Plate II, fig. 1, where the rhizomorphs in the medium are seen as dark blurred areas due to dark hyphae growing out from the rhizomorph. The white spots are young branch initials, consisting of a tuft of floccose hyphae as is produced in nature, which were being produced abundantly when the culture was photographed. This elongation of growing tips is an extraordinary feature and can be carried out at will. The pieces for inoculation are cut from the tips of aerial rhizomorphs formed in culture and laid on the surface of the culture medium. In the course of a day or two the rhizomorph will be seen to have perceptibly lengthened, and still have retained its distinctive characters as a rhizomorph.

III. BLACK LINES PRODUCED IN INOCULATED WOOD.

Armillaria mellea has been grown in pure culture on various wood substrata in a convenient form of culture such as that shown in Plate II, fig. 4. This type of culture consists of a slip of wood placed in a test-tube into the bottom of which is poured a quantity of malt agar. The agar, when it cools, forms a convenient surface for the inoculation of the organism, while its close contact with the wood ensures that the fungus will quickly establish itself in the wood tissues. This form of wood culture has been very successful in raising pure cultures on wood from single spore inoculations. The culture shown in this photograph was derived from the inoculation of a piece of *Armillaria mellea* mycelium on to the malt agar, where the fungus formed a sclerotium-like body at the surface of the medium. Numerous white rhizomorphs can be seen to have arisen from the lower surface of the body and to be spreading throughout the medium. A single rhizomorph, cylindrical and black in colour, can be seen to have arisen from the top of the body and to be growing up the surface of the wood. The rhizomorph is very similar to those found in nature and is giving rise to a number of branches which appear as white tufts, while the discoloration of the wood shows that a certain amount of attack by the rhizomorph has taken place.

In the cultures set up to test the production of black lines by *Armillaria*, larger pieces of wood were used and the inoculum was placed directly on the surface of the wood. Some device to prevent the cultures from drying out too soon, such as cotton-wool soaked in water or a quantity of water in the lower portion of a bulb tube, were included in these cultures which were incubated for a period of 9–12 months. The woods used in the culture were Scots pine and ash and they were inoculated with two separate isolations of *Armillaria mellea*, one from *Veronica* and the other from strawberry[1].

It is evident from observations on the inoculated wood blocks that the mycelium immediately entered the wood at the point of inoculation instead of spreading over the block and giving rise to a mass of superficial mycelium, such as is commonly the case in wood cultures. Subsequent examination showed that the entry was mainly by the medullary rays where there was a considerable number of fine hyphae in the cells.

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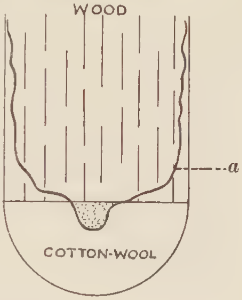
[1] The author is indebted to Mr J. A. Macdonald, Edinburgh and East of Scotland College of Agriculture, for the use of this strain of *Armillaria mellea*. The isolation was made from strawberry plants which had been obviously attacked by rhizomorphs resulting in the formation of broad bands of white fungal plectenchyma in the roots. No black lines or rhizomorphs, however, were observed within the soft tissues of the root.

Thence the spread was in all directions through tracheides, parenchymatous cells and fibres, in the walls of which numerous bore holes could be seen. All the inoculated blocks showed the black lines. Generally, these black lines occurred as the periphery of a large ovoid body occupying the centre of the block (Plate II, fig. 3), but in some cases the lines were quite superficial. A common feature was the formation of a line across the "end wood" some $\frac{1}{16}$ in. below the cut surface. In one such instance (Text-fig. 1) the black line formed beneath the surface of the "end wood" had been extended into the cotton-wool upon which the wood was resting and formed there a structure closely resembling the black xylostroma that is formed below the bark of trees attacked by *Armillaria mellea*. The interior of this structure was composed almost entirely of cotton-wool with a comparatively small bulk of fungal mycelium, whereas the black xylostroma in nature has a solid white medulla of fungal plectenchyma.



Text-fig. 1. Showing the formation of the black line (a) in an inoculated wood block. The black line has been continued into the cotton-wool upon which the block was resting in the culture tube and has formed there a structure comparable to the black xylostroma in nature.

Examination of the culture blocks at varying intervals showed that the method of formation of the *Armillaria* black lines is very similar to that of the *Xylaria polymorpha* lines (5). The position which the line is to occupy is first marked out by aggregations of thin hyaline hyphae. These hyphae swell up to assume the bladder form, and are at first colourless but later become dark brown in colour. The bladder hyphae tend to be formed in patches which spread outwards until they meet with the adjoining patches and thus the complete line is formed. The bladder hyphae are found only in lumina of the cells, and these isolated clumps are connected by a thin hyaline mycelium, which passes from cell to cell by small bore-holes as there is little or no delignification of the cell walls at this time. Indeed, the presence of the bladder hyphae pressed close to the walls so far from bringing about their disintegration actually preserves them, so that, in the later stages of decay, the few sound cell walls are those to which the bladder hyphae have been closely applied. It is important to remember that these bladder hyphae are not continuous but that they occur in clumps which serve to plug the cells in which they are formed. The only connection such a plug has with those in the tracheides around it are by means of a few slender hyphae penetrating the walls. The parts, then, of the tracheide walls incorporated in the line form in the aggregate a considerable bulk of the structure and assist materially in the formation of an impenetrable barrier.

The brown pigment of the bladder hyphae is very similar in reaction to the pigment in *Xylaria*, although the *Armillaria* line in its early stages is not nearly so dark in colour. Again, as in the case of *Xylaria*, a certain amount of pigment escapes from the bladder hyphae, stains the walls of the tracheides and fills the pits between the cells of the medullary rays. The bladder hyphae themselves are rather larger than those of *Xylaria polymorpha*, having an average diameter in the *Veronica* root of about 20μ for the fully developed bladder cell. The average size of the bladder cells, however, is not constant and may vary considerably in different hosts; as, for example, in Douglas fir they are about 18μ in diameter compared with Scots pine at about 24μ. In the case of the bladder cells of the black lines, which were formed in the Scots pine blocks in artificial culture by the *Armillaria mellea* strain isolated from strawberry, a large increase to 35–40μ in diameter was recorded (Plate III, fig. 2). This, however, may be a response to the abnormal conditions pertaining to artificial culture.

IV. SIGNIFICANCE OF THE BLACK LINE.

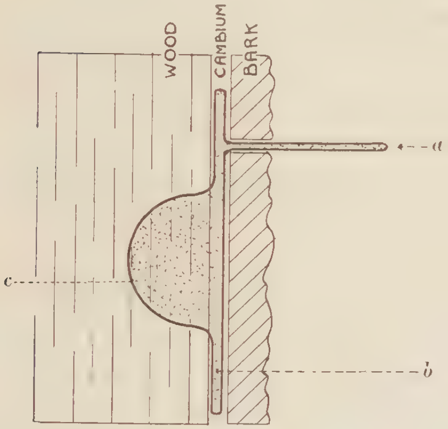
The presence of black lines in wood attacked by *Armillaria mellea* was early recognised, and Hartig (10) in 1878 was the first to describe them. He described the black line as being composed of swollen, bladder-like hyphae, brown in colour, which occupied the lumina of the tracheides just as tyloses are found in the tracheides of many dicotyledonous trees. These bladder hyphae, he stated, were a stage which was highly favourable to the development of the mycelium. In addition, he conceived a sequence of development from hyaline mycelium to bladder hyphae which, when taking place in successive tracheides, would cause the line to advance. He further postulated that the bladder hyphae thus formed soon died off and on digestion were replaced by a delicate filamentous mycelium, and thus the black line (*i.e.* the bladder hyphae in the tracheides) seldom occupied a breadth of more than three or four tracheides. As a result of the passage of the line the walls of the elements of the wood were stated to display a cellulose reaction and speedily dissolve from the lumen outwards.

According to Hiley's account (11) the honey fungus comes to occupy the cambium and from this position invades the wood on one side and the bark on the other. The extent of the invasion in the xylem is marked by a black line, one or more tracheides thick, and generally in the shape of a triangle with its base in the cambium. He came to the conclusion that the bladder hyphae composing the black line represent a stage of excessive vigour in the mycelium which, although it does not directly cause marked changes in the wood, seems in some way to transform the wood into a state in which it is easily acted upon by the hyphae behind. In addition, he further elaborated Hartig's account of the movement of the line and illustrated the mechanism by which this phenomenon was brought about. He differs, however, from Hartig in his statement that no perceptible difference can be seen in the wood on either side of the black line and by pointing out that no more bore-holes are seen on one side than the other, that the tracheide walls give a lignin reaction and that the tori of the bordered pits remain intact. But he states that marked delignification occurs some distance behind the black line and attributes this to the action of the black line.

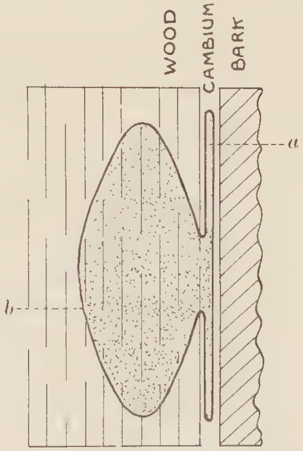
Butler (4) has pointed out that, while the black line may divide the wood into areas that differ in appearance and in the extent of the decay, there is no evidence that the position of the line moves progressively forward. The whole, he declared, is much more like the development of some defensive reaction on the part of the host or parasite.

With regard to the theory advanced by Hartig and Hiley, it must be remarked that if the bladder hyphae are actually "a more active stage in the metabolism of the fungus" then they seem singularly ill-adapted, physiologically, to their function. It is to be expected that hyphae of high functional activity would present the maximum surface area for assimilation and also be well spaced to allow of sufficient aeration. This does not seem to be the case with the hyphae of the black line which are so tightly packed in the tracheide that they immediately suggest a plug (Plate III, figs. 1–3). The writer (5) has demonstrated before how later saprophytic fungi and bacteria can bring about the difference in decay on either side of the black line of *Xylaria polymorpha*, and there can be no doubt that this is also taking place in the case of *Armillaria mellea*. It should also be borne in mind, that in relatively sound wood Hiley was unable to find any difference in the attack on the wood on either side of the black line. In the course of a protracted examination of black lines caused by *Armillaria mellea* in the wood of *Abies noblis*, *Fagus sylvatica*, *Fraxinus excelsior*, *Larix europea*, *Picea excelsa*, *Pinus sylvestris*, *Pyrus aucuparia*, *Pseudotsuga Douglasii*, *Tilia europea*, *Ulmus montana*, and *Veronica propinqua* the writer has in no case been able to discover any evidence of the sequence of events described by Hartig and Hiley which, they stated, resulted in the progressive movement of the line. Nor has the writer found any evidence of these stages in examining material of Hartig's of the black lines of *Armillaria mellea* in pine in the Museum of the Royal Botanic Garden, Edinburgh (Plate III, fig. 1). In all cases the line was observed to be clear cut and distinct and often only a single tracheide in breadth. The walls of the tracheides containing the bladder hyphae are always stained a light brown colour by the pigment from the hyphae so that in this way any movement of the line would be readily detected. Indeed, there is no movement of the line from the position in which it is formed.

The clue to the nature of these lines, however, lies less in their microscopic structure than in their position and in the shapes in which they are found in the substratum. These black lines, of course, are only lines when seen in section and are actually plates or sheets of bladder hyphae. In a transverse section of a root or stem attacked by *Armillaria mellea* the black plates are generally seen in section as black lines or circles, since the plates mostly occur parallel to the tree axis. Again, as Hiley (11) has pointed out, the black lines often occur as a triangle with its base in the cambium. This is, of course, when seen in section, but, actually, the black line is in the form of a shallow basin-like structure with the open mouth at the cambium as is seen in Plate I, fig. 3, and in Text-fig. 2. At the position of the cambium the black plate is continued as a free flattened body, the black xylostroma, which completes the structure. Thus there is formed a body almost completely submerged in the substratum which has for its periphery the black plate or rind of bladder hyphae. This type of body is common at the base of the tree near the point of entry of the fungus, but higher up the trunk the bodies are generally formed deeper in the wood tissues. In this position they are more regular in shape and are seen to be elongated ovoid structures, with their long axis generally parallel to the tree axis, which are buried so deeply in the tissues that only a very small area of their circumference is exposed at the cambium to form the black xylostroma (Text-fig. 3). Such a body in transverse section appears as a black ring of bladder hyphae in the wood as is seen in Plate III, fig. 4. When, however, there is a number of such bodies being formed in the wood their shape is largely determined by the available space, so that generally they become very irregular in size and form. The bodies are all formed quite separately and tend to preserve their individuality to the extent of exerting a repelling influence on each other, so that the two opposing black plates come to be formed always at the same distance from each other, which is usually a few mm., and thus produce the characteristic double or parallel lines. In many cases these bodies may be formed in the bark as well as in the wood, and, when the attack on the cambium has not been severe enough to cause the breaking away of the bark from the wood, the bodies may be formed partly in the bark and partly in the wood. Indeed, since the black plates are formed through all kinds of tissues, a single body may include within it a representative collection of all the tissues of the host.



Text-fig. 2.



Text-fig. 3.

Text-fig. 2. Diagrammatic view of the pseudosclerotium as seen in a longitudinal radial section of the tree. It can be seen to consist of *a*, *Rhizomorpha subterranea*; *b*, the black xylostroma and *Rh. subcorticalis*; and *c*, the body bounded by the black line in the wood.

Text-fig. 3. Diagrammatic view of the pseudosclerotium formed higher up the tree as seen in longitudinal radial section. It consists of *a*, *Rhizomorpha subcorticalis* and *b*, the body bounded by the black line in the wood.

It seems, therefore, that the black plate functions as a limiting layer or rind to the bodies in the wood. As has already been pointed out, this rind, by nature of its closely packed hyphae, presents an impenetrable barrier to the entry of later organisms occupying the same substratum. Another still more important function is that the bounding layer acts as a watertight envelope, which prevents the drying out of the tissues within it, and thus enables the body to withstand conditions of desiccation which would kill mycelium not so protected. Further, the structure of the plate of bladder hyphae is so similar to the rind of the rhizomorph and the wall of many sclerotia that it must be regarded as homologous in character and as forming the rind of a sclerotium-like body, which is immersed in the tissues of the host. In the writer's opinion, the fact that the body is formed almost entirely within the matrix and consists largely of the unaltered tissues of the host does not prevent it from being regarded as a sclerotium, since, physiologically, it serves the same purpose as the more obvious sclerotium. The peculiar nature of a "sclerotium" buried in a woody substratum, however, has led to further developments for the presentation of the reproductive organs at a suitably exposed position and has brought about some remarkable innovations in the character of the "sclerotium" in the wood. The exact nature of these changes and the necessity for distinguishing morphologically this "sclerotium" in the wood from the true sclerotium will be discussed in the next section.

V. NATURE OF THE SCLEROTIUM.

"A fungus, it seems, never begins with a sclerotium; at least we know no fungus whose seeds on beginning to germinate grow at once into so many sclerotia" (Tulasne (14)).

"All sclerotia, it would appear, develope as secondary formations on a primary sporogenous filamentous mycelium" (de Bary (2), p. 34).

The sclerotium, then, is a secondary mycelial condition, following the establishment of the primary mycelium, whereby the reserve food materials are stored in a tuber-like structure to await their utilisation in the formation of reproductive organs. Sclerotia are literally storehouses of food in the concentration of which there is usually a copious exudation of water from the structure. Typically, the sclerotium consists of a uniform white tissue, the medulla, which is surrounded by several layers of large, darker coloured cells which compose the rind. The rind in most cases becomes black in colour, and the mature sclerotium, which has a definite outline and is commonly spherical in shape, generally becomes detached from the mycelium upon which it was borne. Thus the sclerotium is best regarded as a stage in the life history of the fungus, intermediate between the formation of vegetative mycelium and fructification, when the thallus prepares for the reproductive phase. After remaining dormant for a period, the sclerotium germinates by the formation of fructifications of the apothecial, perithecial and basidiomycete types, but the sclerotium of *Botrytis cinerea* produces only simple conidiophores on germination. This is, however, an exceptional case.

The sclerotium may be borne free on the surface of the substratum, or, as is more commonly the case, either wholly or partly immersed in the tissues of the host. In this latter case, pieces of the matrix in which the sclerotium was formed are frequently found within the medulla. Corda (7) has shown that sclerotia like that of *Sclerotinia Sclerotiorum* often enclose portions of the tissues of the plants, which they inhabit, in their own substance, while de Bary ((2), Fig. 19) has figured dead cells of the vine leaf in the sclerotium of *S. Fuckeliana*. *S. Candolleana* may also include parenchymatous cells of the oak leaf within its sclerotium. The presence of host tissues within a sclerotium does not make it necessary to distinguish it as a new type of sclerotium as, indeed, a number of the sclerotia of the Agaricaceae contrive to include within their substance a varied collection of stones and organic debris. The criterion of a true sclerotium is, in the writer's opinion, whether or no the sclerotium is a separate entity with no organic connection with the substratum at the time of germination. Thus the sclerotium, which is borne free on the surface of the substratum or is subsequently set free by the disintegration of the tissues in which it was formed, is a true sclerotium.

VI. THE PSEUDOSCLEROTIUM.

This investigation on black lines has led to the recognition of a number of sclerotium-like bodies in woody tissues. These are very largely embedded in the woody substratum where they are only recognisable by the black lines or plates which comprise the rinds of the bodies. No medulla of fungal plectenchyma is visible to the naked eye, but in its place are the almost unchanged wood tissues of the substratum. Part, however, of this curious sclerotium-like body may be formed on the free surface of the substratum where it has been called the black xylostroma. This black xylostroma immediately suggests on examination its affinity to the true sclerotium; being formed of a white medulla of fungal hyphae and surrounded by a rind of dark-coloured bladder hyphae, which is continued in the wood as a black line or plate. There can be little doubt that this body within the wood fulfils the same physiological requirements as the true sclerotium and, further, there is evidence that the fructifications arise only from such a body or its extension. It differs, however, from the true sclerotium in the perennial nature and curious plasticity of the body which has doubtless arisen from its immersion in woody tissues. It is not advisable, therefore, to include this body in the wood within the term sclerotium on morphological grounds, and, in consequence, it is proposed to introduce the term pseudosclerotium, which will distinguish it morphologically and yet suggest its affinity to the true sclerotium.

The *Xylarias* provide good examples of these sclerotium-like structures formed in the substratum which, in order to establish their connection with similar structures occurring in members of the Diatrypaceae and Diaporthaceae, the writer had previously named entostromata. In many ways the term pseudosclerotium is more comprehensive and capable of a wider application than entostroma to which it must be preferred if only on the grounds that the term stroma has in the past been applied to so many fundamentally different structures. In the case of *Xylaria polymorpha* the pseudosclerotium is almost entirely buried in the wood substratum, but it has the black xylostroma as a free extension beneath the bark. This xylostroma each year develops a number of growing points which grow out from below the bark as the fructifications or "stromata" to bear first the conidia and later the perithecia. These fructifications are simply extensions to and part of the pseudosclerotium in the wood and their structure is very similar to that of the true sclerotium. They have, however, acquired the power of apical growth, which is sufficient to distinguish them from the true sclerotium. This development of apical growth is probably a result of the pseudosclerotium being buried in the wood and the necessity for the reproductive organs being borne at a suitably exposed position on the surface. These fructifications, which arise by apical growth from points on the xylostroma, do not generally grow out more than a few inches, but in culture their length and shape can be considerably modified and, in particular, the exudation of water from the fructifications is strikingly reminiscent of the formation of true sclerotia.

The case of *Armillaria mellea* is parallel to that of *Xylaria*. Again, there is a pseudosclerotium buried in the wood with a free extension of black xylostroma (including *Rhizomorpha subcorticalis*) below the bark from which the rhizomorphs (*Rh. subterranea*) arise by apical growth in a manner comparable to the fructifications of *Xylaria*. The rhizomorphs of *Armillaria mellea*, however, are possessed of the power of unlimited growth, so that not only do they grow out to a suitably exposed position, but they continue to elongate and spread throughout the soil on all sides of their host. But they still remain simply an extension of the pseudosclerotium in the wood and as such still retain the sclerotial function of bearing sporophores. These fructifications may be borne only on the xylostroma, chiefly at the crown of the tree, or, as has been figured by Hartig (10), Hiley (11) and others, on the rhizomorphs in the soil. As a result of the ramifications of the rhizomorphs in the soil, it is inevitable that they will come into contact with the crowns and roots of other plants whose outer cork layers they are able to penetrate as a whole and establish themselves in a new host by giving rise to a vegetative mycelium. This formation of vegetative mycelium is a new method of reproduction for the pseudosclerotium and probably has been developed as a corollary of the unlimited apical growth of the rhizomorphs. The extension of the pseudosclerotium as a rhizomorph, however, is not altogether a new development and is foreshadowed in the formation of the *Xylaria* fructification. Indeed, the rhizomorphs when limited in growth, such as Dade (8) has described as frills of black xylostroma of *Armillaria mellea* on cacao in the Gold Coast, are very similar in appearance to the fructifications of *Xylaria polymorpha*. But it is surprising that this new method of vegetative growth of the pseudosclerotium should be so successful as to oust the sporophore from the position of chief organ of reproduction.

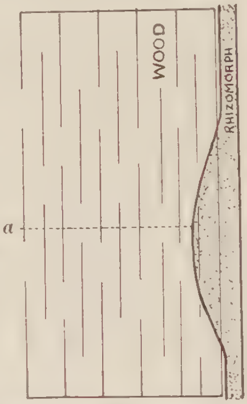
VII. BIOLOGY OF *ARMILLARIA MELLEA* (VAHL) QUEL.

The biology of *Armillaria mellea* so far as it concerns the development of the fungus within the host is not well known. The accounts are incomplete and do not deal fully with the important stages leading up to rhizomorph formation. In addition, the new light thrown on the biology of the fungus by the description of the pseudosclerotia in the wood necessitates a new and fuller account of this phase in the life history of the fungus. So many accounts of *Armillaria mellea* have been published that, with the exception of the pseudosclerotium, the various manifestations of the fungus within the host have already been sufficiently illustrated. As far as possible, therefore, reference will be made to the original descriptions and the writer will only attempt to supply an interpretation of the various stages which have been so described. The fundamental idea behind this interpretation is the distinction between the vegetative and reproductive phases of the mycelium. The vegetative mycelium is composed of thin hyaline hyphae, which bring about the death of the cambium and the delignification of the woody tissues. Following this initial phase comes the stage when the mycelium alters and becomes reproductive in character forming the pseudosclerotia. These pseudosclerotia are adapted for reproduction either by the production of sporophores or by the direct penetration of a new host and the formation of the vegetative mycelium within it.

The methods of infection employed by *Armillaria mellea* have never been made the subject of detailed study although various writers, at one time or another, have stated how they believe the infection to be brought about. It is generally agreed that the basidiospores play no part in the infection of living plants and that they are concerned only in the infection of dead stumps, the mycelium in which eventually produces rhizomorphs capable of attacking living hosts. The rhizomorphs, which are believed to be the main instrument of infection, have been the subject of considerable controversy as to whether they can penetrate the healthy uninjured bark and gain access to the living tree or whether they can only attack through wounds and dead tissues. As long ago as 1877, Brefeld (3) showed that the rhizomorphs could penetrate the living bark of roots, which had been freshly dug up, and thus bring about their infection. Hartig (9) also stated that the rhizomorphs bored into the healthy bark and brought about infection, but Hiley (11) is of the opinion that the rhizomorphs can only enter the host through dead roots and wounds. Recently, Thomas (13) has stated that the invasion of the root is accomplished by the direct penetration of a branch of the parent rhizomorph through the sound healthy cork layer of the host. The method is similar in the various hosts with no apparent difference between susceptible and resistant ones. After gaining entrance, the rhizomorph gives rise in susceptible roots to mycelium which causes the general destruction of the host tissues, while in resistant ones, although the fungus gains entrance, it is unable to establish itself and the wound quickly heals over. In Thomas' opinion the structural and morphological differences in the host exert little influence on resistance, which appears to be in the nature of a vital antagonistic reaction between host and parasite. From observations on young Douglas fir, 6–8 years old, attacked by *Armillaria mellea*, the writer has confirmed that entry and infection in this case were through sound healthy bark, as it was possible by dissection to expose the rhizomorphs where they had grown through the bark and given rise to the mycelium in the cambium. Another method of infection has been recorded from the Gold Coast, where Dade (8), working on a collar crack of cacao caused by *Armillaria mellea*, has stated that the rhizomorphs of the fungus have not been found in nature although they are formed readily in culture. In the cases examined the infection was due to actual contact of the host's roots with the diseased roots of other plants or with rotting wood. He figures the case of an *Eriodendron* root, which had been severed from its parent tree, upon which *Armillaria mellea* was growing saprophytically. Thirty-three yards of this root were uncovered and in its course it was found to have infected six cacao trees by contact with their lateral roots. This method of infection opens up a new field of enquiry, and in this connection more details of the actual infection would be valuable.

Infection, then, is brought about by the rhizomorph penetrating the cork layers of the root as an entity and giving rise to a vegetative mycelium which attacks the cambium. This hyaline mycelium, composed of slender hyphae with numerous branches, spreads rapidly in the cambium, killing the cells and penetrating on either side into the bark and wood. Shortly afterwards a thin white layer of mycelium, papery in texture and marked by ridges, makes its appearance in the place of the killed cambium and begins to spread upwards and downwards in the root. This thin white sheet of mycelium closely appressed to the wood is a very characteristic symptom of the early attack of *Armillaria*, but it is temporary and disappears in the later stages when the rhizomorphs have been formed below the bark. The term xylostroma has been applied to this white mycelial sheet and also to the black layer below the bark, which is the exposed part of the pseudosclerotium in the wood, and it has been assumed that the white xylostroma in time changes into the black xylostroma. Actually this is not the case, as these two mycelial phases are quite distinct. Some parts of the white xylostroma may never change into black xylostroma as is shown in Plate I, fig. 2, where the white mycelium is found alongside the black xylostroma, which has been scraped off to expose the black lines in the wood. On the other hand some portions of the white xylostroma, which have been marked off to form a part of each pseudosclerotium, thicken and eventually develop bladder hyphae until they have formed a complete black rind over the surfaces of the pseudosclerotia. This black rind is continued in the wood as the black plate which delimits the pseudosclerotium. It has been observed in several cases that the black xylostroma and the black lines are always formed more readily where wounds and splits in the bark have exposed the wood and where rapid drying has taken place.

In the examination of the black xylostromata, stages are found where the xylostroma is being resolved into the slender flattened strands or the *Rhizomorpha subcorticalis* of Persoon (Plate I, fig. 4). These rhizomorphs are identical with the xylostroma and are simply an extension of that body where the growing point is confined to the tip of the slender strand. The rhizomorphs by their apical growth spread up the tree following the hyaline mycelium, which has already killed off the cambium and is ramifying in the wood, and occupy the position of the killed cambium between wood and bark. Hyaline hyphae grow out from the rhizomorphs and in this way the rhizomorphs secure a proportion of their food as they ascend the trunk. Sometimes in their passage up the tree the rhizomorphs seem to become partly submerged in the wood, so that the lower part of the rhizomorph is represented by a black plate of bladder hyphae in the wood (Text-fig. 4). Meanwhile, the mycelium farther up the trunk is constantly forming pseudosclerotia, which differ from the lower ones, having a more definite spindle-shape structure and being buried more deeply in the wood. The periphery of such a body appears at the surface of the wood as a long, narrow, black xylostroma, which gives rise directly to a rhizomorph (Text-fig. 3). The pseudosclerotia towards the base of the trunk are shallower or roughly triangular in section, presenting a broad face at the surface of the wood which forms the black xylostromatic mass (Text-fig. 2). In addition to this direct origin of rhizomorphs from pseudosclerotia in the wood, the number of rhizomorphs is considerably augmented by the branching which goes on abundantly if the food supply is sufficient. Altogether this results in a closely woven tissue of rhizomorphs below the bark. Examination of such a weft will not show black lines below all the rhizomorphs. Some, however, will show a definite connection with a pseudosclerotium, if they are carefully examined, but the branching which has been going on has resulted in a number of rhizomorphs which have no obvious connection with the substratum. But it must be remembered that all the rhizomorphs can be traced back to a pseudosclerotium in the wood, however remote it may be.



Text-fig. 4. Showing the formation of a black line representing the rind of a rhizomorph (*Rhizomorpha subcorticalis*) which has become partly immersed in the wood.

Eventually, from the flattened rhizomorphs below the bark, branches grow out between the cracks in the bark to enter the soil, where they do not generally penetrate deeper than a few inches below the surface. These rhizomorphs, the *Rh. subterranea* of Persoon, are now cylindrical with fewer branches and are somewhat smaller than the flattened rhizomorphs below the bark. The flattened shape of the latter rhizomorphs (*Rh. subcorticalis*) has been previously attributed to the narrow space in which they had to develop and to the pressure exerted upon them by the bark. This may be the explanation, but it is interesting to note that in the writer's cultures the rhizomorphs formed within the medium were flattened and band-like. In a very nutrient medium these rhizomorphs attain a size of about 1 in. wide and less than $\frac{1}{8}$ in. thick.

On reaching the surface of the medium these bands assume a cylindrical shape, so that the rhizomorph which projects into the air is always completely cylindrical with a diameter of about $\frac{1}{8}$ in. Observations on these cultures seem to suggest that the flattened shape of the rhizomorph may be due to the expansion of the surface area to allow of increased food intake when in the medium, while outside the presence of air and consequential loss of water by evaporation along with the absence of food to be absorbed will result in the reduction of the surface area to a minimum, which will be met by the formation of a cylinder. On making its way out into the soil through cracks in the bark, the rhizomorph prepares to exercise its reproductive function by the formation of sporophores or by the direct infection of a new host. The remarkable feature, however, of the biology of *Armillaria mellea* is the development of apical growth by which the pseudosclerotium is enabled to bring itself into direct contact with a new host and to implant there its vegetative mycelium. The certainty of this method of reproduction over any development of spore production and distribution is amply emphasised by its success in establishing *Armillaria mellea* as, perhaps, our most serious tree parasite.

VIII. SUMMARY.

The black lines of *Armillaria mellea* have been found in the tissues of a number of plants. Isolations were made from the lines and an account of the development of *Armillaria mellea* in culture is given. Particular mention is made of the formation of sclerotium-like bodies in the culture medium and of the development of the rhizomorphs. Pure cultures of the fungus on wood blocks have produced black lines in the substratum similar to those occurring in nature.

A short review is given of the nature of the sclerotium, and from a consideration of the formation of the black plates, or lines, as they appear in section, the suggestion is made that they form the limiting layer of sclerotium-like bodies, which are partly immersed in the substratum. The black lines in the wood are continued at the surface as the black rind of an effused mycelial mass named the black xylostroma. Thus there is formed a body comparable to a sclerotium in that it is completely surrounded by a black rind of bladder hyphae and has a medulla of hyaline hyphae. It is, however, partly immersed in the wood substratum and, in consequence, contains a considerable proportion of host tissues. Further, this body, probably as a result of its immersion in a hard woody substratum, has developed the power of apical growth resulting in the

free portion of the body growing out into a number of slender flattened strands below the bark of the tree. These strands are the *Rhizomorpha subcorticalis* of Persoon from which, eventually, the more cylindrical *Rh. subterranea* grow out through the cracks in the bark and into the soil. It is proposed to name the body in the wood the pseudosclerotium. This term includes the whole body, namely, the portion within the wood bounded by the black line; the free black xylostroma and its extensions produced by apical growth, the *Rh. subcorticalis* and the *Rh. subterranea.*

A short account is given of the biology of *Armillaria mellea* with particular reference to the pseudosclerotium and its significance in the reproduction of the fungus.

The author is, again, greatly indebted to Dr Malcolm Wilson for his constant interest and valuable criticism. Thanks are also due to Miss I. Lauder-Thomson for the material of *Armillaria mellea* on *Veronica propinqua,* and to Mrs N. L. Alcock for the use of the photograph, Plate I, fig. 1.

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EXPLANATION OF PLATES I—III.

All figures refer to *Armillaria mellea*.

PLATE I.

Fig. 1. Part of the infected shrub of *Veronica propinqua* showing the rhizomorphs (marked by white paper) entering the roots. × $\frac{1}{10}$.

Fig. 2. Six-year-old Douglas fir showing the white xylostroma below the bark. The black lines have been exposed by scraping away the black xylostroma which covered them. A rhizomorph (marked by white paper) can be seen entering the roots. × $\frac{1}{2}$.

Fig. 3. Black circles in the wood of an elm exposed by removing the black xylostroma. Part of the black xylostroma can be seen still attached to the wood. × $\frac{1}{8}$.

Fig. 4. Piece of elm wood showing the ridged and fan-shaped black xylostroma forming slender flattened strands (*Rhizomorpha subcorticalis*). × $\frac{1}{3}$.

Fig. 5. Root of *Veronica propinqua* showing black lines in the wood. × 1.

PLATE II.

Cultural characteristics of *Armillaria mellea*.

Fig. 1. Rhizomorphs formed on malt agar in artificial culture. Some have entered the medium and are giving rise to a halo of dark hyphae. The white dots are young branch initials. Description in text, p. 5. × $\frac{3}{4}$.

Fig. 2. Reverse of cultures on malt-agar slopes showing the formation of a black sheet of bladder hyphae down through the medium to make contact with the glass. As a result the lower part of the slope has not dried out so much as the top portion. Description in text, p. 4. × 1.

Fig. 3. Pine-wood block inoculated with the strawberry strain of *Armillaria mellea* split open to show the black lines in the centre of the block. Incubated for 12 months. × 1.

Fig. 4. Rhizomorph formed in culture growing up the surface of a piece of pine. Young white branches can be seen growing out from the rhizomorph. Description in text, p. 6. × 1.

Fig. 5. Typical culture on oat agar showing the rhizomorphs coming to the surface and the mycelium spreading out from them to join up with the original mycelial plate. Description in text, p. 4. × $\frac{2}{3}$.

Fig. 6. Reverse of culture on malt-agar slope showing the formation of a sclerotium-like body in the depths of the medium. The body, which consists of a black rind surrounding a white medulla, has not developed the rind where it comes into contact with the glass. Rhizomorphs can be seen arising from the body. Description in text, p. 4. × 1.

PLATE III.

Fig. 1. Transverse section through Hartig's material of the *Armillaria* black line in pine showing the packing of the bladder hyphae in the tracheides. Note the clear-cut nature of the line with no signs of the successive stages described by Hartig and Hiley. Unstained. × 250.

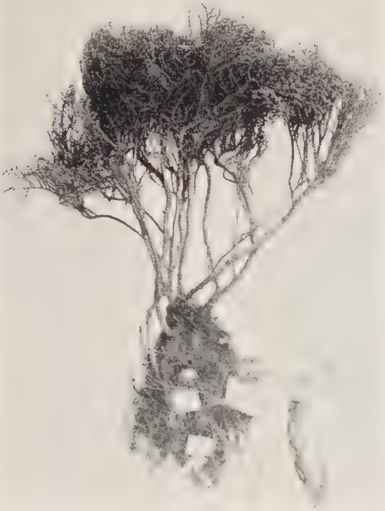
Fig. 2. Longitudinal radial section of pine wood inoculated with the strawberry strain of *Armillaria*. Note the larger size of the bladder hyphae. Unstained. × 150.

Fig. 3. Longitudinal tangential section of black line in pine wood showing the close packing of the bladder hyphae in the tracheides. Unstained. × 150.

Fig. 4. Transverse section of *Veronica* root showing a black circle which represents a section through the pseudosclerotium close to its apex. Unstained. × 60.

Fig. 5. Longitudinal section through the tip of a young rhizomorph formed in culture. The medulla consists of thin hyaline longitudinally arranged hyphae which gradually change into the dark brown bladder cells comprising the rind. At the periphery and especially at the apex is a weft of thin mucilaginous hyphae which are not easily distinguished in the photograph. Unstained. × 80.

(*Received April 26th*, 1933.)



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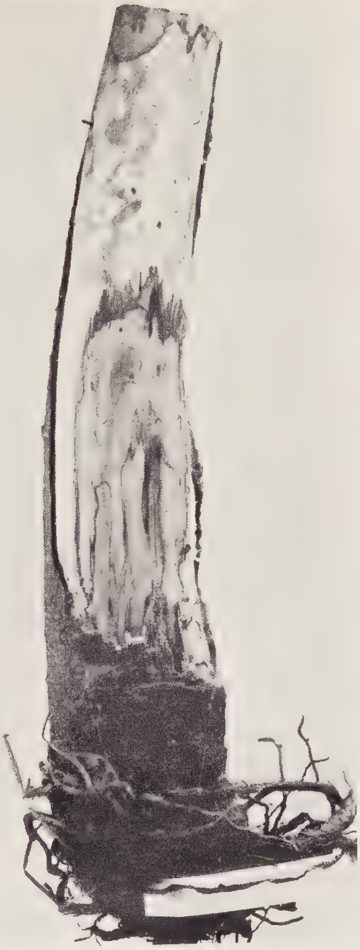

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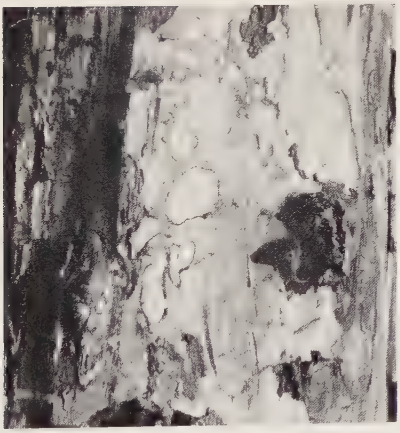

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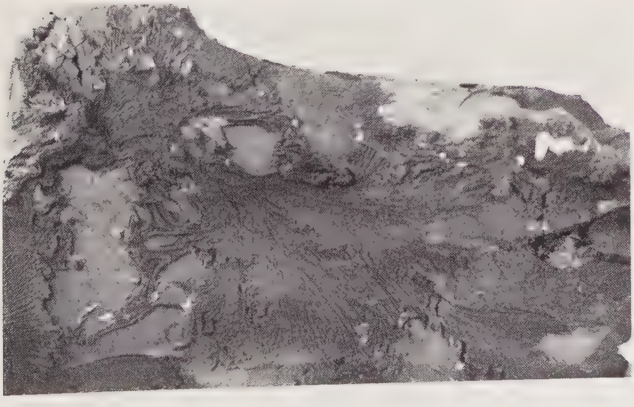

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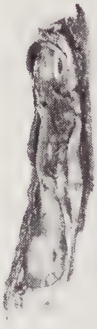
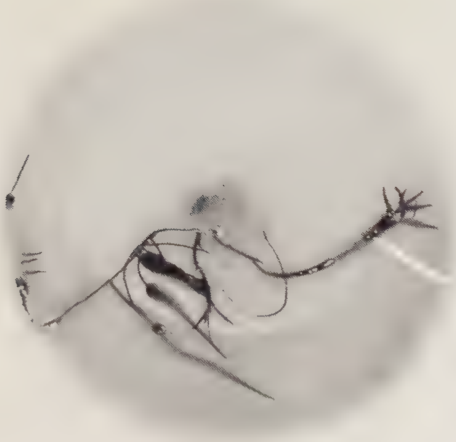


Fig. 5.

CAMPBELL.—Zone Lines in Plant Tissues (pp. 1–22).



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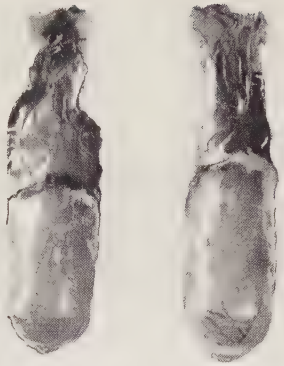

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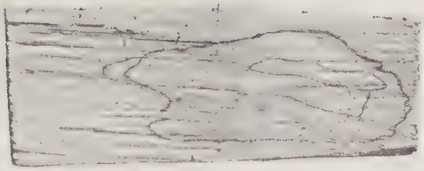

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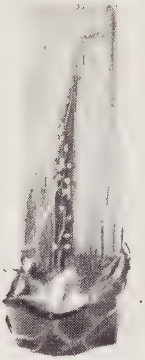

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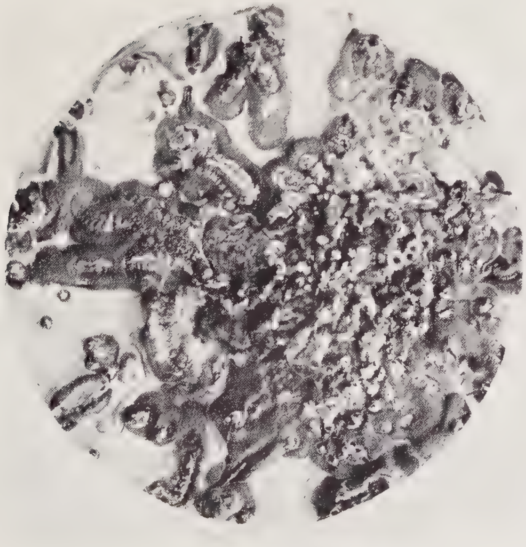

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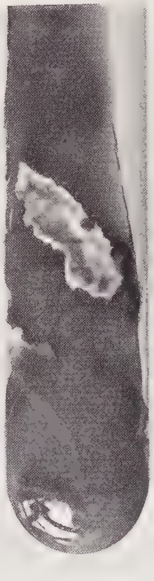
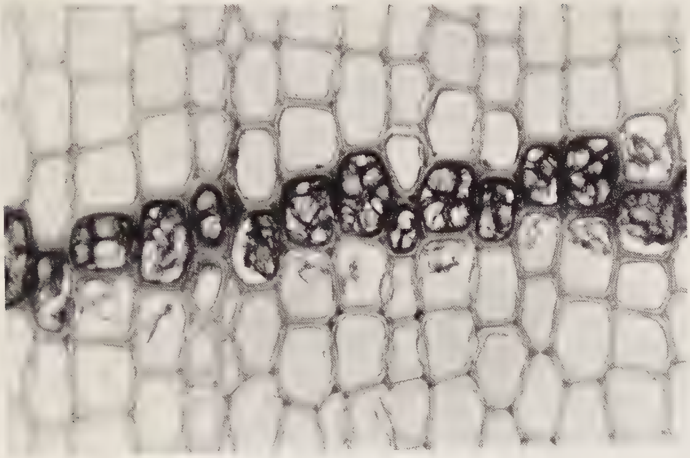


Fig. 6.

CAMPBELL.—Zone Lines in Plant Tissues (pp. 1–22).



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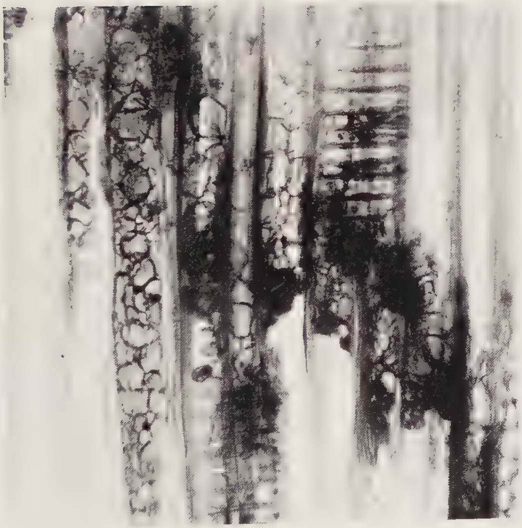

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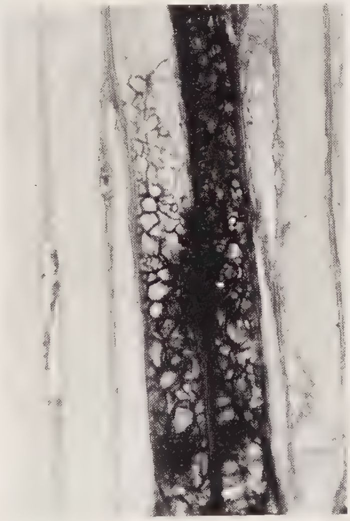

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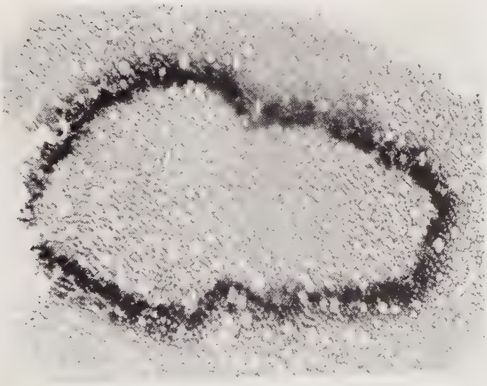

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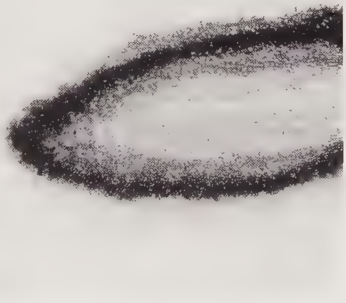

Fig. 5.

CAMPBELL.—Zone Lines in Plant Tissues (pp. 1–22).

ON THE RELATION BETWEEN THE STAGE OF DEVELOPMENT OF THE POTATO CROP AND THE INCIDENCE OF BLIGHT (*PHYTOPHTHORA INFESTANS*)

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I. THEORETICAL CONSIDERATIONS.

It is often stated or implied, both in scientific and in non-technical literature, that potato plants become more susceptible to blight as they grow older. Put in another way, it is a more or less widespread belief that potato plants, when young, are resistant to attack by blight and can become affected only when, or after, they have reached a stage of development that more or less synchronises with the time of flowering. This idea appears to be based on general observations, frequently made, that the earlier planted fields of potatoes go down first and worst in blight epidemics. Indeed, it was expressed almost as soon as epidemics of blight made their first appearance in our potato crops. Thus Mickle (13), writing in 1845, expresses his opinion that "...a certain degree of ripeness or maturity is necessary to feed the disease. Early sorts, from all reports, fall the first victims. Those upon dry, rich, well-drained soils, follow; and late sorts, on cold wet soils, on hill-tops, or with north aspects, that tend to retard growth, seem for a time safe, and flatter the possessor they have escaped; but such no sooner reach the apparently required condition, than they may literally be said to wither in an hour."

Observations on one and the same variety when planted at different dates have been recorded less frequently, and in order to obtain some definite data on the matter a co-operative experiment was planned at the Ministry of Agriculture's Plant Pathological Laboratory at Harpenden and, with the assistance of the provincial Advisory Mycologists was carried out in 1929, and again in 1930, at a number of centres in England and Wales. The purpose of the present paper is to discuss the results of this experiment and of certain other experimental work done in connection with it; but, before doing so, it is considered desirable to deal with some theoretical points and to review briefly the relevant literature on the subject.

A conclusion, which was soon reached, and which may be dealt with first, was that the problem is not a simple and straightforward one, but involves the interplay of several factors, which may vary greatly at different times and places. An epidemic of blight is a complex phenomenon based on two separate events, viz.: (1) the inauguration of one or more sub-aerial primary foci of infection, and (2) the development of secondary infections from such foci and of further successive infections from the ever-increasing new ones.

(1) *Inauguration of the primary foci.* Blight of the overground parts of the potato plant is known to arise from mycelium persisting from the previous season in an occasional, affected, seed tuber. Under appropriate conditions this mycelium gives rise to lesions on some of the shoots, usually not much above ground-level, at which the fungus sporulates. These constitute the primary foci. Primary foci can also arise from affected tubers (or from what may develop from them) that have been left lying about in the neighbourhood of opened clamps or pits. These foci are not necessarily within a new crop, but they are often at no great distance from one; and not at all infrequently they are the source from which epidemics take their origin. The possibility of the occurrence of primary infection from mycelium of the blight fungus over-wintering in the soil, or from "resting" spores of any kind, is not considered here, for there is no satisfactory evidence that infection from such sources does or can occur in nature. In the field, therefore, blight first occurs, as a rule, in not more than a few primary centres, corresponding to blighted tubers or sets accidentally planted; and from these centres further infections arise. Such primary centres are easily overlooked unless most carefully and consistently sought for; the farmer and the casual observer do not notice them. The period elapsing between planting and the production of the primary foci may perhaps be termed the "incubation period" of the disease.

(2) *Development of secondary infections.* These originate from spores produced at and disseminated from the primary centres and are responsible in their turn for the further spread of the disease. It is not to be supposed that there is any sharply defined line of demarcation between primary and secondary infections. Secondary infections, of course, cannot occur before at least one primary focus has become established; but new primary foci may still be produced after the development of secondary infections has started. The rate of spread in a crop will depend chiefly on the weather, since this governs the production of fresh spores to carry on further successive infections and also determines the success or otherwise of infection after dispersal. Under suitable conditions, rapidity of spread will be great, since the number of fresh centres of infection will constantly be increasing. Such conditions, however, rarely predominate all the time; hence the disease will progress in intermittent or undulating fashion rather than with uniformly increasing intensity. This introduces a complication into a "time of planting" experiment, since the different plots mature at different periods, and the plants will therefore not be subject to the same intensity of attack or to the same external conditions at the same age. The rate of spread also depends on the position of the plants in the field, *e.g.* how far away they are from one or more original centres of infection and whether to windward or otherwise.

Theoretically, by planting disease-free seed tubers, it might be contrived that blight should arise and spread in a set of plots by secondary infection only; indeed, it is not impossible that this may occur naturally at times. Unfortunately, in practice it is not possible to ensure this with absolute certainty or uniformity, and the most suitable arrangement that can be devised would appear to be to plant sprouted tubers, that have been carefully examined and found to be healthy, in plots surrounded by a large number of potato plants of the same variety. Sprouting the seed, by enabling apparently healthy tubers only to be selected for planting, will secure a minimum number of primary foci of disease in the plants within the plots; and the larger the number of surrounding plants, the greater will be the chances of uniformity in the subsequent secondary infections.

In this way the rate of spread of the disease in the different plots over a given period can be compared. This gives a measure of what might be termed the "epidemic potentialities" of the plots. This concept is rarely emphasised in plant pathological literature, and it is important to make it clear that it is not the same thing as inherent susceptibility to disease on the part of the individual plants themselves. If 100 inoculations are made on each of two plants under similar favourable conditions and if the number of lesions arising therefrom is 75 and 22 respectively, then clearly the first plant is more susceptible than the second; and if environmental conditions are exactly comparable, we may assess the susceptibilities at 75 and 22 per cent. It is of course assumed here that actual infection is succeeded by the formation of progressively enlarging lesions leading to a blighted condition, as is usual in ordinary potato varieties. If, subsequent to infection, the lesions remained at a standstill in one variety but progressed in another, the latter would probably be regarded as more susceptible than the former, even if the number of lesions was the same in each case. Other methods of measurement are, of course, available, and may not give exactly the same figures, but as comparative figures only come into consideration the principles are the same. The susceptibility arrived at in this way is the *inherent susceptibility* and may be expected to vary somewhat with individuals in the same way as other characters do. In any collection of plants the susceptibilities will vary about a mean which may be termed the "mean susceptibility" of the group. This is quite different from epidemic susceptibility, perhaps better called "epidemic potentiality" which, although dependent, of course, on inherent susceptibility, does not bear a simple relation to it, but also depends on the proximity of the plants to each other, on the number of spores available for infection, and perhaps especially on variable external conditions, particularly those existing amongst and around small groups of plants; in a word, on the "micro-climate" as envisaged by Roussakov (25). Complications also arise owing to the cumulative effect of successive infections and to the discontinuous character of an epidemic. In general statements about the greater susceptibility of older plants epidemic potentiality is really implied, although its distinction from inherent susceptibility is not generally appreciated. When the effects of a difference in epidemic potentiality, such as may exist in different fields or plots, become evident, it cannot be concluded that a difference in inherent susceptibility exists unless it is certain that the external conditions (including the micro-climate) have been the same and that the number of fungal infections (or number of centres of infection per unit area) have also been the same.

The idea that young potato plants are less susceptible to blight than old ones is difficult to reconcile with the fact that young seedlings and sprouts often succumb very readily to infection. When potato plants are raised from seed from the "apple," and when the young seedlings are exposed, under favourable conditions of atmospheric humidity and temperature, to infection with the blight fungus, by atomising them with a spore suspension in water, most of them quickly become infected and succumb to the disease. Those that remain healthy have presumably some degree of inherent resistance to the disease. In any case there can be no question here of escape through immaturity, since all the seedlings are of the same age.

Again, provided atmospheric humidity and temperature are suitable, there is no difficulty in artificially infecting with blight the healthy young sprouts first arising from potato tubers. Müller (17) states that all ordinary varieties are equally easy to infect artificially in the young sprout stage. The seasonal recurrence of blight epidemics results from the preliminary development of the primary infection foci during what has already been alluded to as the "incubation period," and these are due to the ability of young tissues to become attacked by the fungus. Another fact that may be cited in favour of the inherent susceptibility of young tissues is the observation of Reddick (23) that the very resistant variety Ekishirazu can be infected artificially, but only in the very young leaves at the tip of the stem, and the resulting lesion spreads down the stem only a short distance; in field-grown plants of this variety leaf lesions have not been observed. Even on the susceptible varieties commonly grown, the first attacks of blight noticeable by the ordinary person are not infrequently on the youngest tips of the shoots rather than on the older leaves. Hence there seems to be no *a priori* reason for attributing any special power of resistance to the young organs of the plant.

The few varieties of potato in the British Isles that at present show a very high degree of inherent resistance to blight happen to be rather late ripening sorts such as Champion II, Irish Chieftain and Shamrock. They are best known in Ireland, although not at all commonly grown even there, as their quality is rather inferior. They do not appear to be known on the Continent or in America. It is, however, begging the question to assume, as has been done[1], that freedom from attack in these varieties is due, not to inherent resistance in the variety, but merely to escape from infection owing to immaturity when the blight epidemic is at its height. Perret (19), indeed, records the fact that Violette de Forez, although a late variety, is highly susceptible to blight, both in foliage and tubers, whereas the earlier variety, l'Industrie, is highly resistant. Moreover, it has been demonstrated that the tubers of Shamrock show inherent resistance to artificial infection (Pethybridge (20)); the same thing would probably hold good for the other varieties mentioned, although it must not be assumed that resistance in the tuber is necessarily accompanied by resistance in the foliage or *vice versa*. The notion (O'Connor (18)) that amongst our domestic varieties of potato there is none that possesses inherent (lately styled "genetic") resistance to blight seems contrary to fact; and it has yet to be proved that the resistance to blight found in rare instances in wild species of tuber-bearing Solanums is something of a totally different character from that shown in a few domestic varieties.

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[1] *E.g.* by Salaman, *2e Congrès Internat. Path. Comparée*, 1931, II, 435. Champion (*i.e.* Scottish Champion, not Champion II) was resistant to blight fifty years ago, but is now one of the most susceptible varieties in cultivation.

II. PREVIOUS INVESTIGATIONS.

From what has been said it will be seen that there is no *a priori* evidence in favour of resistance to blight being a characteristic feature of the young parts of potato plants as opposed to the older parts. The more direct evidence that has been put forward in support of this idea must now be examined. The observations may be grouped under three headings: (1) general field observations; (2) experimental plots planted at different times; (3) artificial inoculation experiments. It will be shown that the first two admit, in reality, of deductions about epidemic potentiality only; for information about inherent susceptibility investigation on the lines of the third heading is essential. In the accounts of different authors' results which follow, the word "susceptibility", when used unqualified, is employed in the same sense as that intended by the author concerned.

Mickle's (13) remarks, published in 1845, have already been referred to, but perhaps Kühn (11), in 1871, was the best known phytopathologist to call attention to apparent differences in susceptibility to blight in potatoes planted at different times. According to Miss de Bruijn (6), Kühn believed that the potato plant showed two periods of susceptibility, one in the very young stage and another later, separated from the first by a period of vigorous foliage development characterised by resistance to blight. In potatoes of the same variety, planted partly early and partly late in 1864, it was observed at the time of lifting that the overground parts of the early plants had been destroyed by blight whereas those planted abnormally late were still fresh and green.

In concluding the account of his researches on the nature of the potato fungus, written in 1875, de Bary (2) cautiously says: "From large experience, I consider it probable that *Phytophthora* grows more easily on a plant at the height of its development than on young stalks and leaves. It would be interesting, but not easy, to establish this clearly by experiment."

An anonymous writer (probably Sorauer) refers (1) to an observation made by Büchner in 1894 (confirming others of previous years) that the spread of blight appears to depend on the attainment of a definite stage of development of the foliage of the individual varieties. When blight ordinarily sets in, the early varieties have usually reached the susceptible stage and thus Büchner saw them severely attacked, whilst the mid-earlies were only slightly attacked and the late ones not at all. In another season, weather favouring blight did not occur till later, and then the foliage of the late varieties had reached the susceptible stage and was completely blackened. In one place, however, where the tubers were planted very late, the plants retained their green foliage.

In 1898, Hecke (9) maintained that we are not entitled to assume differences in susceptibility of the overground parts of a potato plant to blight at different periods of its life, although he did suggest that the incubation period of the fungus itself, *i.e.* the time taken to sporulate after inoculation, might perhaps vary with the age of the inoculated leaves or stems.

In his discussion of disease resistance in potatoes, published in 1905, L. R. Jones (10) refers to what he calls the "critical period" in the development of the potato plant, which arrives just after the plant has come into blossom. "It is noteworthy," he says, "that the destructive attacks of the late-blight fungus occur, as a rule, after the blossoming period has passed. So far as the evidence goes, it seems to suggest that high vegetative vigour enables the plant to ward off in some degree the fungus attack."

Eriksson (8), in 1917, noted that blight occurred in Sweden, under natural conditions, late in the season, whereas in plants grown under forced conditions it appeared early. This led him to believe that the occurrence of blight is not connected with the season of the year, but with a certain stage of development of the plant, namely that at which vegetative growth, *i.e.* the production of stem, leaf and inflorescence, has reached its terminus; only after that can blight secure a foothold on the plant.

Whilst carrying out investigations on wart disease in Holland during the years 1922–4 Botjes (5) paid some attention to the occurrence of blight in his plots. He found it very difficult to express the time of maturity of a variety as a definite date, or the degree of *Phytophthora* infection of the foliage in figures, since in many cases it was almost impossible to decide whether death of the plants was due to natural ripening or to attack by blight. He states that *Phytophthora* infection is regulated to a high degree by the state of ripeness of the plant. In a plot of Eigenheimer, in which the tops of some of the plants were removed, the new shoots that first came above ground in July remained normally green until October and showed merely a few dark spots. The foliage of the untouched remaining plants, however, was completely cut down by blight at the beginning of August.

Mielck (14), as a result of field observations in Germany in 1926, when he saw early varieties attacked by blight whilst the late ones remained free, came to the conclusion that young plants are intrinsically more resistant than old ones; and Perret (19) in the following year, from observations made in France, stated that in one and the same variety susceptibility to blight appears to increase step by step with the advance of the plant towards maturity.

Müller (15, 16), in the course of his work in search of resistant varieties of potatoes, makes numerous observations on the problem. He concludes that high resistance of the young plant is not the cause of the relatively late appearance of blight, but that the latter is due to the fact that mass attacks cannot take place early in the season owing to unsuitable external conditions. Nevertheless he believes that potato plants become more susceptible to attack when they approach maturity, although not necessarily because of any changes in the internal constitution of the host. It seems probable that here the author is really thinking of epidemic potentiality.

All the above observations do not take account of the fact that the external conditions in the comparisons made are not the same. The blight fungus is very sensitive to variations in temperature and humidity, either of which may become a limiting factor. Unless it is ensured that the conditions are the same, one cannot be certain that the observed differences are not to be explained by differences in the environment.

Turning now to the experimental side of the subject, Pethybridge (20, 22), in 1909, appears to have been the first to have endeavoured to ascertain by field experiment whether young plants are more resistant to *Phytophthora* attack than older ones. For this purpose a series of plots of potatoes was arranged in the west of Ireland (where external conditions favourable to blight are the rule rather than the exception), in which planting was done at intervals of from 7 to 14 days, beginning on March 11th and ending on August 9th. The name of the variety used is not stated, but is believed to have been Scottish Champion, one highly susceptible to blight. The plots were not immediately contiguous to other potatoes planted at the normal time, but other plots of such potatoes were within a few yards of them at the temporary experimental station where the trial was made. Blight appeared first on July 11th, not on the oldest plants but on those in two plots planted on the 19th and the 26th of April respectively. It then spread more or less rapidly over the other plots in a manner quite irrespective of the ages or developmental stages of the plants. Plants arising from tubers set at the end of July and early in August reached a height of only 2 or 3 in. before they became attacked and destroyed by blight. It was concluded that youth or immaturity of the plant did not confer resistance to attack.

In a chapter on the influence of the stage of development of the potato plant on its susceptibility to infection with blight Miss Löhnis (12) stated that in Holland in 1919 blight appeared late in the season, and simultaneously on early and late varieties in the same field. In the following year plots were planted at monthly intervals during April, May and June, and in that season the youngest plants showed the first blighted leaves five days later than the control plants. The trial was regarded as incomplete, and the conclusion arrived at was merely that "it is probable that the stage of development of the plant has an influence on the moment of outbreak" of the disease. On the other hand, working in the field with artificial methods of infection, Miss Löhnis found it possible to infect potato plants with blight three weeks before the natural outbreak occurred, and secondary infections following such primary artificial infections proceeded quite as rapidly as in a normal epidemic. It was concluded, therefore, that "the stage of development of a plant has no influence on the chance of contagion through neighbouring plants."

Incidental to an attempt to produce a high-yielding variety of potato, immune from blight, Collins (7) studied the physiological aspects of the incidence of the disease during the years 1916 to 1921. Basing his analyses on what he terms the "apparent correlation" between resistance to blight and the time of maturity of the plant, he endeavoured to determine what changes in the physiological condition of the foliage accompanied maturation. He came to the conclusion that the degree of susceptibility of a variety to blight is indicated rather precisely by the water : nitrogen ratio, a value which increases during the season and, in general, is highest at the time of infection. Young foliage was found to have a lower water and a higher nitrogen content than foliage of medium age, whilst in old foliage the reverse conditions held. Unfortunately, no details are furnished as to how the experimental plants were grown, to what conditions they were exposed, or how infection was secured. Hence for the purpose of deciding whether young plants are inherently more resistant to blight than old ones the published account gives little help, and the correlation must remain but an apparent one.

In 1923 and 1924 Miss de Bruijn (6) made plantings of six varieties of potatoes each fortnight, from the middle of March to the middle of August. In the first year blight did not appear until August 10th, when it was first seen in two of the earliest (March) planted plots. In 1924 it appeared first on July 23rd, and not in the early (March and April) planted plots but in those planted later, viz. in May. In the August planted plots, in 1923, the shoots came above ground at a time when blight had already been present a considerable time, and the date of incidence on these plots was not noted. When examined, however, a few spots of blight were to be seen on all the young plants. In the August 1924 planted plots, however, the young plants came up when an epidemic was at its height, and the weather was extremely favourable to blight. The young plants immediately became very heavily attacked, and one variety was completely wiped out by the disease. In spite of this, from a study of the progress of the disease in the plots, the authoress concludes that her results confirm the view that the foliage of the potato plant is not at all times equally susceptible to blight attack. An analysis of the data published in her paper does not support her conclusion. By plotting curves it can clearly be seen that the rate of increase of blight did not vary very much in different plots. The later plantings succumbed later, but the first infections were later, so that the rate of spread was not less than in the earlier plantings. Actually, in some varieties, the rate was greater, owing no doubt to the weather being more favourable at the later date.

These experiments by Pethybridge, Löhnis and de Bruijn appear to be the only time of planting trials so far carried out except those of Schilberszky to be alluded to later and those to be described in this paper, already partially reported on elsewhere (3, 4). It will be seen that Pethybridge is the only writer (with the exception perhaps of Hecke and Müller) to have doubts as to the correctness of the opinion that susceptibility to blight is less in young plants than in old ones. His experiment certainly does not support this opinion, but it is possible that the absence of differences in epidemic potentiality between the different plots was due to environmental conditions. No data being available it is not possible to ascertain this: but it seems probable that the epidemic was severe in that year, and that differences in incubation chances did not exist.

Theoretically, artificial infection experiments should solve the problem of the existence or otherwise of any relation between susceptibility to attack and a definite age or stage of maturity of the plant, but the published results on work of this kind are conflicting.

In 1926 Vowinckel (27) in Germany made a few experiments on the influence of the age of the potato plant on the development of blight. Plants that were started in pots on the same date were inoculated with the fungus in batches (beginning on the 28th day) at intervals of a week. After inoculation the plants were kept under bell jars for a short period and then exposed in a north window, under conditions that were necessarily not constant throughout the experiment. In plants four weeks old 60 per cent. of inoculations succeeded; five weeks, 80 per cent.; and later, 90–100 per cent. One impression gained was that the spread of the fungus in early infected very young plants was slower than in the later infections of the older ones. In another experiment a number of plants was artificially infected (1) at flowering time (between July 27th and August 20th), and (2) on September 29th. The weather was said not to be more favourable in the later infection. Three days after inoculation it was found, on the average, that 17 per cent. of the first infections and 42 per cent. of the second had resulted in sporulation. From these experiments the author concluded that the potato plant can be attacked by *Phytophthora* in every stage of its development, but a difference in the behaviour of the parasite can be recognised in plants of different ages. Towards the end of its period of growth the plant is more susceptible, at any rate from the time onwards when the overground organs have completely developed, and very probably also at an earlier period than this. Vowinckel also artificially inoculated very young, full grown and quite old leaves of individual plants of thirty-three varieties, and measured the areas of tissue killed after four days. The average areas were in the ratio 29 : 47 : 53, from which he concluded that leaves become more susceptible with increasing age, and that the leaves of different ages on one and the same plant at a given moment show different degrees of susceptibility.

This conclusion appears to be confirmed by some observations of Reddick (23). He noticed in some of his plantings certain very large plants which, in late summer, had reached a condition in which the lower very much shaded leaves turned yellow, and dropped. After a particularly heavy natural infection with blight in 1926 some of these plants showed no blight lesions, or only very small spots, on the green foliage, whilst the senescent leaves were attacked, and the fungus sporulated on them, though not in definite lesions. Reddick rightly considers that these general observations require more specific investigation.

A weakness of Vowinckel's experiments lies in the impossibility of being certain that the external conditions were exactly comparable throughout. It is well known that quite small changes in humidity, for example, may have a marked effect on the progress of blight. This difficulty may be got over by carrying out the inoculations at the same time and place on healthy plants planted at different dates, a considerable number being planted together so that the environment can be considered uniform. Such an experiment was carried out by Schilberszky (26), who refers in his account of the ecology of the potato fungus, published in 1928, to experiments carried out by him in 1916 to ascertain the effect of the age of the plant on artificial infection with the blight fungus. Three varieties were used, and batches of each of them were planted on April 25th, May 6th and 15th. On June 18th all were sprayed with water containing spores of *P. infestans.* Blight was noted first on June 27th. On this date it was distinct in the earliest planted lot, some of the plants of which showed incipient flower buds. It was only sparingly present in the intermediate lot and nearly absent from the last planted one. The experiment was repeated with slight modifications in the following year when, eight days after spraying with a spore suspension, the two earlier planted lots showed about equal moderate infection, whilst the third showed much less. From these trials Schilberszky concluded that it may perhaps be inferred that the progress of *Phytophthora* infection stands in a definite connection with the degree of development of the plants, and he suggested that this might perhaps bear some relation to chemical differences in the sap at various ages of the plant.

Quite different results were obtained in experiments carried out by Miss M. E. Hough at Seale-Hayne College in 1930. King Edward plants that had been set on May 19th, June 14th and 30th, and July 14th respectively within a large bed of potatoes were all artificially inoculated with the blight fungus on August 6th. All the plants showed equal amounts of infection within three days. King Edward is a very susceptible variety, and it is possible that under conditions favourable to the disease such a variety might not show variations in attack corresponding to age, whereas a less susceptible one might perhaps do so. On the other hand it is possible that the conditions in Schilberszky's experiments were not uniform; for example, he sprayed his plants with water containing spores and this may not have resulted in uniform fungal infection. In Miss Hough's experiments the inoculations were specifically localised and were marked with Indian ink. Whatever may be the explanation of the disagreement, it is evident that many further experiments are required to give us clear information on the problem.

To summarise the above discussion of previous investigations, it has been shown that general field observations indicate a greater *epidemic potentiality* in older plants but give no satisfactory evidence of any change in inherent susceptibility. The evidence for the latter is too circumstantial to be of very much value. Experiments on the dates of planting of plots exposed to natural infection have not given any reliable indication of any differences in either inherent susceptibility or epidemic potentiality. The results of artificial inoculation experiments are equally unreliable.

The question may be asked, How is it possible to have differences in epidemic potentiality if inherent susceptibility remains the same? It must be remembered that the disease arises both by primary incubation and by secondary infection. First of all, assuming the number of primary foci to be the same, the rate of progress would be expected to be greater in the older plants, since the number of possible secondary infections will be greater, owing to the larger number of leaves. It is also probable that the number of primary foci will be larger in the older plants, since there will have been a longer time available for them to come into existence, during which time favourable conditions for their development will have occurred. Periods of high humidity and sufficient warmth are necessary for the development of primary infection foci and for the production of spores at them, and successive periods probably exert a cumulative effect. The chances of both primary and secondary infection are greater the older the plant up to a point, for the "micro-climatic" conditions, especially those of humidity, are likely to be more favourable to the development of the fungus in and around older plants with well-developed foliage than in younger, smaller ones more exposed to the drying influences of sunshine and air currents. On such grounds, quite apart from any difference in inherent susceptibility, one would expect the disease to appear first in the oldest plants, and in them a greater degree of epidemic potentiality will exist.

III. THE DATES OF PLANTING CO-OPERATIVE EXPERIMENT IN 1929.

The variety King Edward was used, except at centre 13 (Bangor), where British Queen and Up-to-Date were partly employed. The seed was obtained from different sources. Plots were laid down at the following centres under the superintendence of the advisers named.

Centre	Adviser
1. Harpenden, Herts	A. Smith
2. Cambridge	Dillon-Weston
3. Berkshire	Buddin
4. Lincolnshire	Stirrup
5. Newton Abbot, Devon (3 series)	Beaumont
6. Heddon, Northumberland	Bennett
7. Penrith, Cumberland (2 series)	Bennett
8. Nantcellan, Cardiganshire	Davies
9. Hutton, Lancs	Holmes Smith
10. Long Ashton, Somerset	Ogilvie
11. Newport, Salop (2 series)	Preston
12. Cardiff, South Wales	Rees
13. Bangor, North Wales (3 series)	Whitehead

Plantings on eight different dates at fortnightly intervals were made at each centre except Nantcellan, where there were nine, and Heddon and Long Ashton, where the number in each case was seven. A minimum of twelve tubers was planted in each plot, and the plots were surrounded on all sides by potatoes planted at the ordinary time. The successive plantings were arranged so as not to adjoin one another directly, but according to a plan such as the following, the numbers representing the successive dates on which planting took place, 1 being the earliest and 8 the latest:

7	5	4	2
1	3	6	8

At Heddon and Bangor the plots were situated in the middle of fields of potatoes. At Hutton the plots were within a distance of 200 yards from a farm crop of potatoes. At Cardiff they were situated amongst plots of lucerne and sainfoin, while at the other centres potatoes were planted around the plots, the minimum width of this surround being 6 ft. Such an arrangement is not quite as satisfactory as when the plots are in a large field of potatoes, since initial infection is more likely to come from one direction than from all quarters.

Blight was not serious in 1929; it was late in appearing and did not occur at all at the first four centres. The observations made at the other centres are summarised in Table I. At some of the centres the plots were in duplicate or in triplicate and this is indicated by the letters *a*, *b*, *c* after the number of the centre.

Table I.

Centre	Date of first planting	Blight first noted	Plots attacked at first observation
5 *a*	Mar. 14	—	—
5 *b*	„ 14	July 12	2, 4
5 *c*	„ 14	Aug. 23	5, 6, 7, 8
6	May 5	Sept. 2	1, 2
7 *a*	Apr. 5	July 28	1, 2
7 *b*	„ 5	„ 30	1, 2
8	„ 20	Aug. 24	2
9	„ 1	„ 27	2, 3
10	„ 15	„ 24	All
11 *a*	„ 18	„ 23	1, 2, 6
11 *b*	„ 18	„ 23	1, 2, 3, 4, 6
12 *a*	„ 5	„ 16	2, 3
12 *b*	„ 5	„ 16	2, 3, 4, 5, 6
13 *a*	„ 2	„ 27	1, 3, 4
13 *b*	„ 2	„ 27	3
13 *c*	„ 2	„ 27	6, 7

The subsequent progress of the disease varied considerably in the different localities. At centres 6, 7, 9, 10, 11 the observations definitely indicated an increasing resistance to epidemic spread among the younger plants. At centre 8 (Nantcellan) all plants were attacked within seven days, so that they appeared to be equally susceptible. At centre 12 (Cardiff) the epidemic was equally rapid, but the observations indicated a more rapid development of decay in the older plantings. The epidemic was very rapid also at centre 13 (Bangor), and the distribution of the disease was rather irregular. The experiment 13 *c* was, however, complicated by the necessity of using three varieties, owing to the difficulty of getting a supply of King Edward seed. The first four plantings were of British Queen and the remainder of Up-to-Date. Blight appeared earliest in the first two plantings of the latter, *i.e.* plots 6 and 7. It spread to all the other plots within three days. At centre 5 (Newton Abbot) the disease appeared earlier than elsewhere but made very slow progress, passing from one plot to the next without regard to the age of the plants. The soil of the plots varied considerably in depth, and the spread of blight was down the slope in the direction of increasing depth. The effect of shallow soil in causing earlier maturity may have masked to some extent the effect of time of planting, while the relative positions of the plots have also to be taken into account.

In considering the above results two facts must be borne in mind:

(1) blight was everywhere late in appearing so that the plants in the three first planted plots were probably all equally mature at that time, at any rate, at those centres where planting started early; (2) the epidemic, once it had begun, spread very rapidly, so that in the absence of daily observations the exact sequence of attack would be missed.

Further, it is not easy to interpret the significance of the results without a knowledge of: (1) the exact position of the plots, relative both to each other and to the focus of infection; (2) the severity of the epidemic; (3) the degree of maturity of the plants at the beginning of the epidemic; and information on these points is unfortunately not available for all centres.

All that can be concluded from the 1929 series of experiments is that in a late attack of blight the tendency is for the older plants to become blighted first. Subsequent infections may be equally rapid on all plots or may be least rapid on the younger plants, according to weather conditions and to the relative positions of the plots.

No conclusions could be drawn as to variations in individual susceptibility unless it could unequivocally be ascertained whether the disease arose first as a primary infection focus originating from a diseased tuber, or by secondary infection. Assuming it arose in the first way, this would presumably occur, following the law of probabilities, in the oldest plots first, and the epidemic potentiality of these plots would be greatest (without there being any differences in individual susceptibility) because the plants in them would be nearest to the primary centre of infection. If the disease had arisen by secondary infection from all quarters, and if the "micro-climate" had been the same in all cases, the epidemic potentialities of the different plots would then be directly proportional to individual susceptibility, and the experiments would tend to show that the older plots had greater inherent susceptibility. The nature of the lay-out of the experiments, however, prevents such a conclusion from being drawn, for at many of the centres secondary infection certainly was not uniform. Sufficient data are not available to say with confidence exactly how the disease arose in the first place at each centre.

IV. THE DATES OF PLANTING CO-OPERATIVE EXPERIMENT IN 1930.

In 1930 the seed, variety King Edward (at all centres except Bangor where one set of plots (5 *c* below) was of British Queen), was distributed from a common source in Northumberland. Plots were laid down at the following centres:

Centre	Adviser
1. Harpenden, Herts	A. Smith
2. Hutton, Lancs	Holmes Smith
3. Heddon, Northumberland (2 series)	Bennett
4. Penrith, Cumberland	Bennett
5. Bangor, North Wales (3 series)	Whitehead
6. Sutton Bonington, Leics	Stirrup
7. Newport, Salop	Preston
8. Cardiff, South Wales (2 series)	Rees
9. Long Ashton, Somerset	Ogilvie
10. Reading, Berks	Buddin
11. Newton Abbot, Devon (3 series)	Beaumont

Eight plantings were made at fortnightly intervals at each centre except Sutton Bonington (six), Newport (seven) and Cardiff (nine). They were arranged irregularly as in the previous season, except at Newport, where all the tubers on each date were planted in one continuous row.

The plots were in the middle of a farm crop of potatoes at centres 3, 6, 8; 100 yards west of a potato crop at centre 2; in a cultivated orchard at centre 4; and were surrounded by specially planted plots of potatoes at the other centres.

Blight was again late in appearing, but was more severe than in 1929. The observations at each centre are summarised in Table II.

Table II.

Centre	Date of first planting	Blight first noted	Plots attacked at first observation
1	Apr. 16	Sept. 20	All except 1 which had died down
2	,, 1	July 25	1, 2, 3, 4
3 *a*	May 12	Aug. 26	1, 2
3 *b*	,, 12	,, 26	1, 2
4	Apr. 24	,, 17	1, 2
5 *a*	,, 14	,, 21	1, 2, 3, 8
5 *b*	,, 14	,, 21	1, 2, 4, 6, 8
5 *c*	,, 14	,, 19	1, 2, 3, 4
6	,, 12	End of August	—
7	May 3	Aug. 8	1, 2, 3, 4, 5, 7
8 *a*, *b*	Mar. 18	,, 5	—
9	Apr. 28	,, 13	—
10	,, 15	,, 11	—
11 *a*	,, 7	,, 4	1, 2, 3, 4
11 *b*	,, 7	,, 2	1, 3, 5
11 *c*	,, 7	,, 3	1, 2, 7

At centres 2, 3, 4, 6, 7 the observations made showed a greater rapidity of spread in the oldest plants. The results from Cardiff (8) are not included, as half of each plot was sprayed; the first observation at

Long Ashton (9) was not made till September 12th, when the worst attacked plants were the oldest; at Reading (10) no differences were observed. At Harpenden (1) the disease did not appear until all the plants were mature. The early appearance of blight on the most recent planting (plot 8) at Bangor (5 *b* and 5 *c*) was due to its proximity to plot 2, the most heavily attacked plot. The inclusion of the second-early variety British Queen (5 *c*) in the trials also upset the uniformity of blight infection.

Series 11 *b* and 11 *c* at Newton Abbot were on the same site as 5 *b* and 5 *c* of the 1929 experiments. The same explanation again applies to the somewhat irregular distribution of blight as in the previous season, and will be discussed in the light of the details given in the next section.

As in the previous year's trials, it is impossible to say with certainty whether the disease first arose primarily within the plots themselves or by secondary infection from without. The probability is that it started primarily in the oldest plots and the greater epidemic potentiality in the latter then follows as a matter of course. No conclusive evidence is, however, available on this point.

V. OBSERVATIONS AT SEALE-HAYNE COLLEGE IN 1930.

The results at the Newton Abbot centre referred to in the previous section can now be discussed more fully, since the present writer was in personal charge of the plots. At this centre it was possible to make detailed observations of the extent of the disease in each plot every day. The number of plants affected and the number of lesions on each plant were counted, and the results were plotted on squared paper. The two sets of data gave the same results, and to illustrate them Table III is given which shows the total number of lesions in each of the eight plots in each series, up to a certain date (August 15th), and then the estimated percentage degree of attack on each plot as a whole.

For certain dates the number of lesions recorded is less than that for the previous count; this is because the affected parts had either dried up and fallen off or had been eaten by snails (*Helix virgata*), which were present in considerable numbers.

The delay in the appearance of blight in plot 2 of series 11 *b* was probably due to its position being the farthest away from the first centre of infection. With this exception the above data show that during the first week of attack there was, on the average, a lag behind plots 1 and 2 of four days, the later plots not catching up until the foliage was completely attacked. Plots 3–8 showed a smaller difference amongst

Table III.

Date	Plot							
	1	2	3	4	5	6	7	8
			Series 11 *a.*					
Aug. 4	11	10	1	1	0	0	0	0
5	25	28	4	7	2	1	1	3
6	39	16	7	6	5	1	0	2
7	44	37	5	5	3	1	0	0
8	41	34	5	6	5	1	0	1
9	68	82	9	16	3	2	4	0
10	Many	Many	127	131	60	8	34	7
11	—	—	Many	206	141	26	67	19
12	—	—	—	*c.* 500	260	104	173	62
14	—	—	—	*c.* 500	260	104	136	62
15	—	—	—	*c.* 500	260	119	155	73
26	100%	100%	90%	95%	90%	75%	90%	70%
29	100%	100%	100%	100%	100%	95%	100%	95%
31	100%	100%	100%	100%	100%	100%	100%	99%
			Series 11 *b.*					
Aug. 2	3	0	1	0	1	0	0	0
4	3	0	5	1	1	0	0	0
5	22	5	7	4	1	0	0	0
6	35	6	7	9	1	0	1	0
7	43	13	4	2	1	0	1	0
8	57	17	11	3	3	0	2	0
9	67	14	29	13	8	1	2	2
10	Many	30	153	65	43	19	30	15
11	—	39	Many	79	66	32	65	15
12	—	51	—	150	97	63	89	24
14	—	65	—	128	84	59	68	39
15	—	115	—	144	185	57	166	61
26	99%	85%	95%	80%	80%	65%	80%	75%
29	100%	100%	100%	100%	100%	100%	100%	100%
			Series 11 *c.*					
Aug. 3	1	1	0	0	0	0	1	0
4	0	1	1	0	0	0	1	0
5	0	2	0	3	2	1	1	0
6	6	3	2	1	2	2	2	0
7	9	3	1	2	3	2	1	0
8	8	5	1	2	5	4	3	0
9	13	5	2	1	3	2	1	0
10	15	19	2	15	5	13	5	3
11	31	49	10	41	17	38	6	3
12	73	Many	30	51	20	77	21	9
14	67	124	50	79	54	108	34	20
15	171	Many	92	81	78	161	47	28
26	80%	95%	65%	60%	35%	50%	50%	30%
29	Each plot 95–100%, except plot 8, 70%							

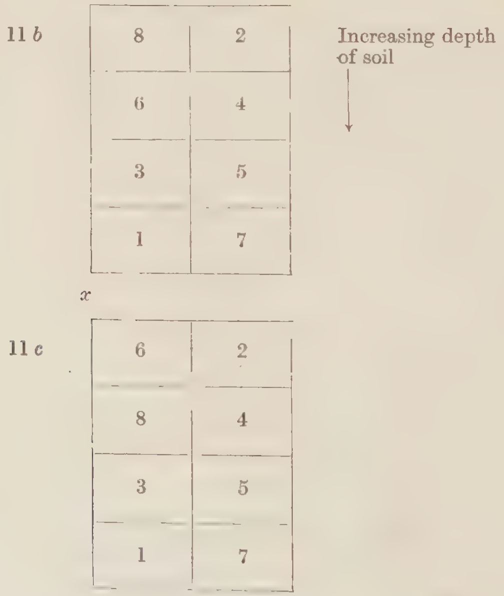
themselves, each consecutive pair of plots being only about a day in front of the next.

This is summarised in Table IV.

Table IV.

	Plot							
	1	2	3	4	5	6	7	8
Dates (August)			*Series 11 a.*					
About 10 plants attacked	5	5	9	9	10	10	10	11
About 20 plants attacked	7	7	10	10	10	11	11	13
All plants attacked	8	8	13	10	11	14	13	14
			Series 11 b.					
About 10 plants attacked	5	10	9	9	10	10	10	11
About 20 plants attacked	7	11	10	10	11	11	11	14
All plants attacked	8	11	11	11	11	11	11	15
			Series 11 c.					
Over 10 plants attacked	9	10	12	11	12	11	14	14
Over 20 plants attacked	11	11	12	12	12	12	15	15

In other words the rate of progress of the disease up to the same dates is at first greater in the oldest plants. On subsequent dates, however, the rate of progress on even the youngest plant may be as great as or even greater than that amongst the old plants at the earlier date. The two governing factors are weather conditions and the number of initial infections, and it is impossible to separate these factors in an experiment of the present type.



It will be noted that the plants in series *c* succumbed much more slowly to blight than those in *b*, although situated only 3 yards away.

The same phenomenon was observed in the previous year's experiment and was ascribed to the greater depth of soil in series *c*. The accompanying plan of the plots shows their distribution in relation to depth of soil and original centre of infection (x).

VI. FURTHER OBSERVATIONS AT SEALE-HAYNE COLLEGE IN 1931.

The experiment was repeated once more at Seale-Hayne College in 1931, when the variety King Edward was again used, the seed being from Northumberland. Two series of plots were planted on the following dates:

	Plot							
	1	2	3	4	5	6	7	8
Series A	Mar. 24	Apr. 7	Apr. 21	May 5	May 19	June 2	June 17	June 30
Series B	Apr. 22	May 6	May 20	June 3	June 10	June 17	June 24	July 1

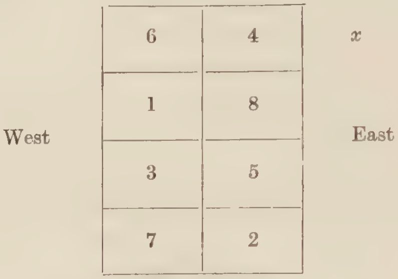
The plots in series A were close to two of the previous year's plots (11 *b* and 11 *c*), but those in B were in fresh soil that had been waste ground in which potatoes had never previously been grown. There were forty-two plants in each plot arranged in seven rows. The plots in both series were surrounded by other potatoes as in the previous experiments.

Blight appeared in some of the plots of series A before all the plantings had been completed. On June 11th blight lesions were observed on 22 plants in the headland rows adjoining plot 4 in this series and they increased in number rapidly. Plot 4 first became attacked, three plants showing the disease on June 12th. The numbers of attacked plants recorded each day are shown in Table V.

Table V.

	Plot							
Date	1	2	3	4	5	6	7	8
June 15	1	—	2	6	—	—	—	—
17	1	—	1	11	—	—	—	—
19	—	1	1	18	1	—	—	—
20	1	1	1	19	—	—	—	—
21	1	3	1	21	—	—	—	—
24	—	8	1	36	8	—	—	—
25	—	10	1	27	14	—	—	—
28	37	36	26	40	All	28	—	—
30	40	All	38	40	All	32	—	—
July 5–11	All	All	All	All	All	All	—	—
13–17	All	All	All	All	All	All	All	—
18	All	All	All	All	All	All	All	All, though only 1 in. high

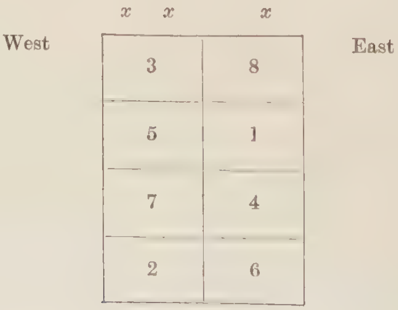
It is clear, therefore, that the progress of blight was not according to time of planting but depended on the proximity of the particular plots to the original source of infection, as shown in the following diagram (source of infection indicated by x).



The disease spread slowly from plant to plant and more quickly down the rows than across them. The prevailing winds were westerly, which would tend to check the spread of the disease to the plots on the left. It is interesting to note that the plants in plot 7 did not appear above ground till July 5th, and those in plot 8 not till July 13th, by which time they were completely surrounded by blighted plants. All the plants in each of these two plots developed blight simultaneously.

This experiment shows very clearly the importance of the location of the source of origin of the fungal infection as a factor governing epidemic potentiality. The rapidity with which the youngest plots succumbed showed that under conditions of maximum fungal infection and favourable environment their epidemic potentiality was very great. This is exactly what was observed by Pethybridge in his experiments in 1909(20), and, as pointed out by him, certainly does not indicate any increased inherent resistance in young plants.

In series B blight was not so early in appearing, but its progress was equally rapid. It began on July 11th in the headland plants above plots 3 and 8 (at the spots marked by x in the diagram below) and was seen on July 13th in plots 1 and 2.



There were between sixty and sixty-four plants arranged in seven rows in each plot. The records of the numbers of plants attacked each day given in Table VI show that blight developed in the plots in fairly close relation to the times of planting.

Table VI.

	Plot							
Date	1	2	3	4	5	6	7	8
July 13	5	2	—	—	—	—	—	—
15	30	2	18	18	—	—	—	—
17	All	All	All	40	8	2	—	—
19	—	—	—	42	All	38	All	7
22	—	—	—	All	—	All	—	All

The surrounding headland infection was much more nearly uniform than in series A, except that there was a large amount of disease to the west of plots 5 and 7, which explains the abnormal rapidity of the attack in these plots.

VII. CONCLUSIONS FROM DATES OF PLANTING EXPERIMENTS.

It will be evident from the foregoing account that a simple field experiment in which the results of natural infection are kept under observation is not capable of being sufficiently rigidly controlled to give definite information on individual susceptibility. The results are, however, of value in that their analysis enables a clearer conception to be obtained of the progress of an epidemic. They do show that in the majority of cases the epidemic potentiality of a group of mature plants is greater than that of a similar group of young plants. Differences in the direction and amount of fungal infection and in the environmental conditions will cause differences in epidemic potentiality; and it is not necessary to assume the existence of any changes in inherent susceptibility correlated with the ages of the plants. The existence of such a correlation has certainly not been proved.

VIII. SUMMARY.

The evidence for the common opinion that potato plants above a certain age are on that account more susceptible to blight than younger ones is based on (1) general field observations, (2) dates of planting experiments, (3) artificial inoculation experiments. The results of such observations and experiments are discussed and summarised, and it is shown that they do not consistently support this opinion; further, it is pointed out that much more careful analysis than is usually employed is needed to interpret the results correctly.

The difference in behaviour towards blight sometimes observed between assemblages of old and young plants appears in reality to be due to variation in "epidemic potentiality," and not to change in inherent susceptibility with age. It is not necessary to assume the existence of differences in susceptibility between plants of different ages, for the differences in disease intensity and resultant rate of spread will follow from the increasing chances of the occurrence of primary infection foci as the plants become older, and from the greater chances of the environment (particularly the micro-climate) being suitable for the development of the parasite amongst older than amongst younger plants.

Accurate conclusions concerning inherent resistance or susceptibility are possible only from carefully controlled artificial inoculation experiments carried out under uniform optimum infection conditions. Seedlings are often readily attacked so that youthfulness does not confer any degree of resistance on the plant. The results of experiments hitherto carried out with older plants are conflicting, and further study of the problem on carefully planned lines is eminently desirable.

The author acknowledges the very great assistance given by Dr G. H. Pethybridge in the preparation of this paper and in surveying the literature. The inception of the experiments was due to him and he has been in close touch with the work from the beginning. Acknowledgment is also tendered to those Advisory Mycologists who co-operated in the date of planting experiments, and whose records of observations the author has been enabled to consult and utilise.

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THE PHYSIOLOGY OF POTATO LEAF-ROLL

I. ON THE RESPIRATION OF HEALTHY[1] AND LEAF-ROLL INFECTED POTATOES

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(With 20 Text-figures.)

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I. INTRODUCTION.

The obstacles in the way of determining the aetiology of virus diseases have so far proved unsurmountable and, in consequence, the diseases can only be studied in relation to the reaction of the infected plant. The most obvious reaction, except in the case of "perfect carriers," is a more or less severe morphological modification, so it is not surprising that considerable attention has been paid to this aspect of the problem. The present attempts to analyse symptom complexes form a logical development of morphological studies, and are useful in enabling more or less accurate labels to be affixed to certain morphological reactions of the plant, but it is not sufficiently recognised that the clinical picture presented by a virus-infected plant is only the outward manifestation of metabolic disturbances. This is particularly evident in the potato, in which the variation in the clinical expression of different varieties infected with the same virus, as well as the change within the same plant at different stages of growth or under different environmental conditions, clearly indicate the fundamental importance of physiological studies.

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[1] *I.e.* normal in appearance, with no implication of complete freedom from viruses "carried" without symptoms.

Preliminary work over a number of years with potato leaf-roll had indicated that the infected plant deviates markedly from the normal in several physiological functions including respiration, and this latter function was selected for detailed study as being most susceptible of accurate measurement whilst being at the same time highly responsive to metabolic disturbances. The object of the work was to trace the effect of the disease on the respiration of the potato at all stages in its life cycle, so that it was of course necessary to carry out a parallel investigation with normal plants. The work of Kidd. West and Briggs at Cambridge (4) is the only other attempt, known to the writer, to trace out the course of respiratory activity throughout the life of a plant. The present work differs not only in being a comparative study of diseased and healthy plants, but also in being concerned with a vegetatively reproduced perennial in which problems of dormancy and the prolonged dependence of the plant on the reserve food of the seed tubers are involved. A preliminary account of this work appeared in *Nature* in 1931 (11).

II. MATERIAL AND METHODS.

Tubers of the variety Arran Comrade were obtained from field crops in 1930; the infected ones being from a stock which had shown the disease since 1921, whilst the healthy tubers were obtained from a crop maintained since 1922 under relatively isolated conditions on the College mountain land at an altitude of 1000 ft. Some of these healthy stocks were planted under lowland conditions in 1931 and formed the source from which immature tubers were detached in July and August of that year, care being taken to make use of plants as far as possible removed from sources of infection. A succession of growing plants was provided by planting tubers at short intervals of time in pots under aphis-free glasshouse conditions. In some cases the dry-matter content of the tubers was determined immediately after an experiment, but in others the tubers were grown on so that the respiration could again be determined at a later stage in the life cycle.

The method adopted for the measurement of the rate of respiration was the collection of the CO_2 in Pettenkofer tubes containing standardised $N/10$ baryta, which was then titrated against similarly standardised $N/10$ HCl. In the earlier part of the work, tubers, in groups of from six to twenty, were enclosed in an electrically controlled thermostat chamber kept at 20° C., whilst single growing plants had their roots, in pots, under these controlled conditions with the single-stemmed shoots enclosed in a bell-jar at a temperature about 2° C. below that of the roots. The shoot was sealed off from the roots by a perforated glass plate, the hole through which the stem passed being first plugged with cotton-wool overlaid with plasticine, on which was cemented a thin rubber disc tightly enclosing the stem and itself covered by a thick rubber disc cemented down with plasticine; the stem between the rubber discs was thickly coated with grafting wax. There was sufficient resilience in the discs to permit growth in length and girth whilst maintaining a perfect seal. In the case of the growing plants a difficulty arose in that to maintain a constant temperature the thermostat root chamber had to be sealed off from the outer air, and measurements of respiration could only be taken over a period of about 48 hours to avoid root-asphyxiation effects. In one experiment the respiration (in oxygen and in nitrogen) of whole plants (healthy and diseased) including the seed tuber and roots, was determined in the thermostat (Fig. 8), but in all other cases the term "respiration of the growing plant" means that of a single-stemmed shoot properly sealed off from the root system and soil.

During this earlier work comparable healthy and leaf-roll material followed each other in the one apparatus, but the bulk of the experiments were carried out in parallel sets of apparatus, the one containing healthy and the other diseased material. This involved the discarding of the thermostat respiration chamber, since only one was available, but enabled the experiments to be prolonged indefinitely (usually for about 120 hours) with both plants under identical conditions and with the roots freely exposed to the air; the advantages so gained more than compensating for the loss in uniformity of temperature. An initial stabilising period required in the earlier work owing to the use of a bell-jar as a plant container (cf. Figs. 7, 8 and 9), was also eliminated by devising a respiration chamber constructed of glass cylinders adjustable in height, so that the minimum capacity consistent with proper freedom of the plant to grow could be obtained. The details of this adjustable chamber are described in Fig. 1. In work with single tubers and with very young detached shoots, the respiration chamber consisted of a "Copenhagen" bell-jar, just sufficiently large enough to contain the plant material, and in this case also no stabilising period was required.

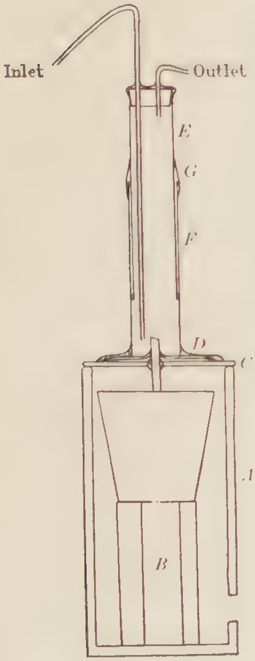

Fig. 1.

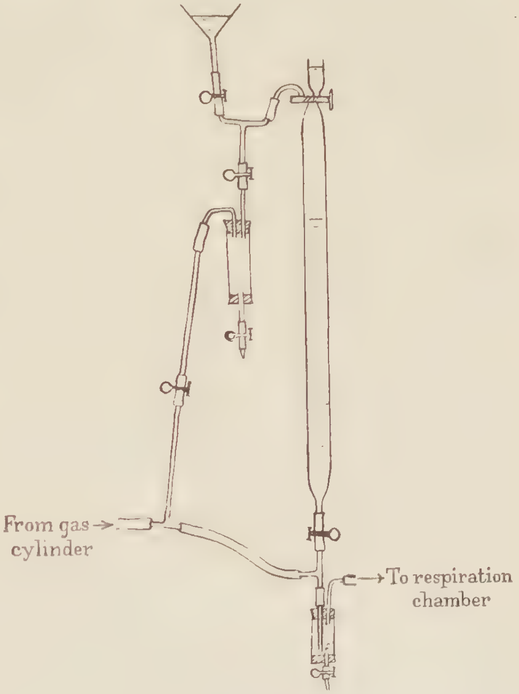

Fig. 2.

Fig. 1. Adjustable plant chamber. *A*, earthenware jar with tubulure. *B*, support for plant-pot. *C*, perforated ground-glass plate cemented to *A*. *D*, rubber discs and plasticine for sealing off the foliage in plant container *EF* from the roots and external air. *E* and *F*, glass cylinders, the former sliding within the latter and sealed at the top with plasticined rubber stopper through which passes inlet and outlet glass-tubes. *G*, strong rubber band allowing adjustment of foliage chamber whilst maintaining a perfect seal.

Fig. 2. Apparatus for ensuring complete absence of oxygen throughout the duration of anaerobic experiments. Essentially a closed system within the respiration apparatus and comprising a Schiff's nitrometer used for mixing potash and pyrogallic acid in the absence of oxygen; the resulting straw-coloured liquid being used as required in the lower trap through which passed a stream of nitrogen to the respiratory chamber.

Co_2-free air was drawn through the apparatus, by means of a Maryott bottle aspirator, at the rate of 500 c.c. per hour, except in the case of single tubers when this rate was reduced to 200 c.c. per hour. The air was deprived of its CO_2 by passage through a potash tower containing pumice over which there was a constant flow of 50 per cent. caustic potash; this arrangement preventing any head of pressure developing. Before entering the respiration chamber the air was bubbled through 10 c.c. of baryta to ensure the complete removal of CO_2, and then passed over boiled distilled water to maintain a reasonably constant moisture content of the chamber. From the respiration chamber the air passed through 50 or 100 c.c. of baryta in a Pettenkofer tube, according to the intensity of respiration anticipated, then through a further 20 c.c. baryta trap and finally over a sensitive mercury trap to ensure that the whole apparatus remained at atmospheric pressure. After some preliminary testing and standardising of the apparatus, and particularly of the exit tube of the aspirator, a considerable degree of constancy in the rate of flow of water was obtained, some 12 hours being required without adjustment before the rate of flow was reduced from 500 to 480 c.c. per hour.

Anaerobic work was carried out in an essentially similar apparatus. Nitrogen was supplied in a compressed gas cylinder and was stated to have the composition of 99·5 per cent. nitrogen, 0·15 per cent. helium, 0·05 per cent. neon; the remaining 0·3 per cent. being mostly oxygen. A steady rate of emission of the gas, at all events until the pressure within the cylinder became seriously reduced, was ensured by means of a gas regulator fixed to the cylinder nozzle. The gas was bubbled through two sealed jars of pyrogallol to absorb the 0·3 per cent. oxygen present. Before entering the 10 c.c. baryta trap the gas passed through a solution of pyrogallol prepared in the absence of oxygen in the following manner. A Schiff's nitrometer was inserted in the apparatus as shown in Fig. 2, and was filled with boiled distilled water. Potash and pyrogallic acid, contained in the two receptacles above the two-way tap, were admitted alternately at the same rate as the water was run out at the base. Eventually the burette was thus filled with a mixture in any proportions desired, the resulting solution being of a pale straw colour extremely sensitive to the presence of oxygen. Any leakage in the apparatus was sufficient to darken the colour of the pyrogallol appreciably, so that the complete absence of oxygen throughout the duration of an experiment could be guaranteed. In setting up an experiment the regular succession of drops of water from the aspirator to give a rate of 500 c.c. of air renewed per hour was first obtained, and the inlet tube was then connected to the pyrogallol jars and so to the gas cylinder. The emission of gas was then regulated to give the same rate of flow from the aspirator so that the plant was exposed to a pressure of nitrogen equal to that of atmospheric air. When, as was usually the case, aerobic respiration was followed by anaerobic, the transition from oxygen to nitrogen was made as abruptly as possible. The plant chamber was flushed out with several times its volume of nitrogen, so that, apart from its own internal oxygen content, the plant was placed at once under anaerobic conditions.

There remains only to discuss the method used in stating the results. In many cases the same material was grown on after one experiment in order that the respiration could again be determined at a later stage in the life cycle, so that the results could only be expressed in terms of the fresh weight in the initial experiment. Wherever possible, however, both fresh weight and dry weight were used as a basis and the method used in no way affected the conclusions arrived at. To save space, therefore, all results have been recorded only in terms of the fresh-weight basis, except in the case of plants periodically exposed to light when the results are stated in terms of dry weight in Appendix V and in Figs. 16–19, and on the basis of fresh weight in Fig. 20.

III. RESPIRATION AT SUCCESSIVE STAGES IN THE LIFE CYCLE.

A. *Dormant tubers.*

(i) *Immature tubers.* The respiration of these tubers was in every case determined in the paired apparatus, single tubers being contained in chambers constructed from "Copenhagen" bell-jars provided with inlet and outlet tubes. The two aspirators drew CO_2-free air from the same potash tower at the rate of 200 c.c. per hour. This apparatus was also used in other work with single tubers and the agreement between the two halves of the apparatus was tested repeatedly, two examples of which are illustrated in Fig. 6 where two healthy *or* two infected tubers occupied the respiration chambers at the same time.

The term "immature" is usually applied to tubers which have had less than the normal period in the soil. They are readily recognised by the thin papery skin, the difficulty with which they are detached from the stolon, and by the fact that the foliage is still green and vigorous. As thus defined, immaturity can be obtained either by early lifting or late planting. Although it is not known whether the chemical composition of the tubers is affected by the method used, the visible reaction is identical in tubers from plants in which the growth has been restricted to the early part of the year and those which began their growth in late summer.

Most of the immature tubers used in the present work were raised in early August, and the respiration was determined at once and again after storing for periods varying from three weeks to three months at room temperature. During any one experiment there was little fluctuation in the rate of respiration (cf. Fig. 3*b*), but a progressive decline occurred according to the length of the storage period. Appendix I shows that the rate of respiration immediately after lifting in August was about 0·0180 mg. per hour per gram fresh weight for healthy tubers and 0·0275 mg. for diseased ones. After storing for one month these had fallen to 0·0080 and 0·0107 mg. for healthy and diseased respectively, whilst after three months' storage the comparable figures were 0·0038 and 0·0046 mg. In all the experiments the leaf-roll tubers consistently showed the higher rate of respiration. During July (*i.e.* some three weeks prior to the beginning of the experiments referred to above) very immature tubers with thin papery skins were raised from rather moist soil and the rate of respiration determined after they had been kept in wet silver sand for three days. The initial rate was higher than in the case of the August raised material (0·0866 mg. for healthy and 0·1003 mg. for diseased tubers), and differed also in showing a rapid fall until stabilisation was reached in three or four days at 0·0279 and 0·0292 mg. for healthy and diseased tubers respectively. This somewhat spectacular fall in the rate of respiration could be repeated in the same tubers by careful removal of the skin. It was not a wound reaction, since it occurred when little or no abrasion of the skin had been caused, but is readily explainable as being due to the very rapid loss of water through the thin periderm. That water readily passes through the skin of young developing tubers is demonstrated by their ceasing to swell when they are separated from the soil moisture by means of dry sand enclosed in a cardboard cylinder; the stolon being quite unable to supply sufficient water for normal growth. In the above experiments the tubers were much drier and firmer at the end of the experiment than at the beginning (Fig. 3*a* and Appendix I, Exp. 1).

(ii) *Mature tubers.* By the middle of September the haulms had ripened off and tubers were raised on the 28th and used both at once and again after storage for about five weeks. During this period the rate of respiration fell from 0·0074 mg. per hour per gram fresh weight to 0·0066 mg. in the case of the healthy tubers, and from 0·0094 to 0·0077 mg.

for diseased ones. Tubers raised in October and kept in storage for five months were used in three experiments and showed a still further reduction in the rate of respiration to 0·0037 mg. for healthy and 0·0033 mg. for diseased (cf. Appendix I, Exps. 8–10). The figures given

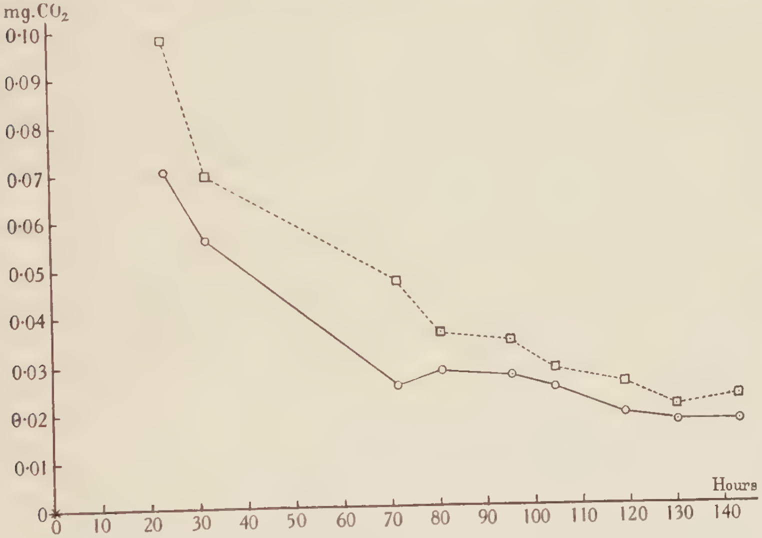

Fig. 3*a*. Immature tubers from moist sand in July, and after peeling. Healthy and leaf-roll shown as continuous and dotted lines respectively.

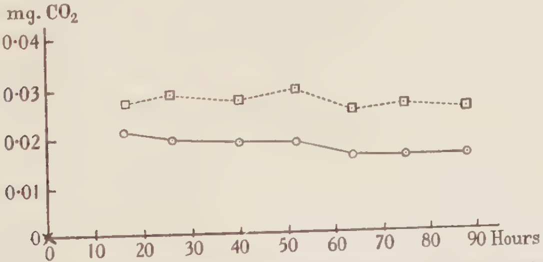

Fig. 3*b*. Immature tubers immediately after lifting in August. Healthy and leaf-roll shown as continuous and dotted lines respectively.

for the last experiment are averages of three experiments which closely agreed with each other. They each consisted of groups of from six to twenty tubers kept at a constant temperature of 20° C. (as described under Section II) during the determination of the rate of respiration.

The respiration of mature dormant tubers under anaerobic conditions was determined after storage for three weeks and gave a rate of 0·0054 mg.

for healthy and 0·0061 mg. for diseased ones (cf. Fig. 4, and Appendix VI, Exp. 1). The respiration in nitrogen, therefore, was similar to, but a little lower than, that for similar tubers under aerobic conditions.

(iii) *Summarising dormant tubers.* To summarise the position as regards tubers in a dormant condition: mature tubers immediately after raising show a definitely lower rate of respiration than do immature tubers straight from the field (Appendix I). With both mature and immature tubers there is a decline in the rate depending on the length of the storage period, and the final level reached is very similar in both cases. The infected tuber, whether prematurely raised or not, shows the higher rate of respiration until the end of the rest period approaches when, if anything, it has a slightly lower rate than the healthy tuber. Although there is an appreciable loss of water during storage, this cannot explain the fall in the rate of respiration, as in the case of the immature tubers from damp silver sand, for McLean has shown that healthy tubers lose water very much faster than do leaf-roll infected tubers (6), and this would tend to keep the rate of respiration of diseased tubers at an increasingly higher level as the storage period was prolonged. Nor is it conceivable that the fall can be due to any lack in the fundamental substrate, starch. Appleman and Miller (1), working with (presumably) healthy American varieties of potatoes, also found immature tubers to respire at a higher rate than fully mature ones, but that after storage the rate was the same for both classes. Their explanation is based on the higher ratio of sucrose to reducing sugars in tubers still making rapid growth (*i.e.* immature tubers), but it is clear that further work is necessary in view of Barton-Wright and M'Bain's recent statement that the details of carbohydrate metabolism in the *foliage* differ in healthy and leaf-roll infected plants (2). It is along these lines that an explanation must be sought of the failure of the diseased tubers to maintain the higher respiration throughout the rest period.

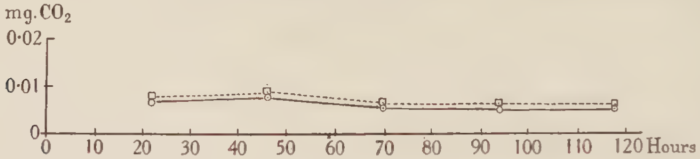

Fig. 4. Anaerobic respiration of mature dormant tubers after storage for three weeks. Six tubers used in each case. Healthy shown with continuous line; leaf-roll with dotted line.

Apart from the question of virus infection, and so far as respiration can be accepted as a criterion, prematurely raised tubers mature normally in storage and are almost indistinguishable from those lifted when mature, after storage. The writer, therefore, agrees with Appleman and Miller that maturation is a continuous process, beginning in the field and continuing during the rest period. Quite different results have been obtained with ripening fruit, *e.g.* Blackman and Parija with apples (3), and Magness and Ballard with pears (5). In such cases a period of rising respiration occurs after removal of the fruit from the tree during senescence, followed by a decline as starvation effects predominate. The rise is explained by the former workers as due to a lowering of the "organisation resistance," which is plainly not to be seen with maturing tubers, and the term "ripening" or "maturation" should not be applied both to organs which have reached the end of their function and those about to begin a new phase of active life.

The termination of the rest period and the rate of development of the sprouts are determined by the conditions of storage and not by the degree of immaturity when the tubers are raised. The writer found in two varieties (British Queen and King Edward VII) that tubers raised on successive dates from July to September showed a downward grading of the size of sprouts developing from those with the longest to those with the shortest period in storage. On the other hand, tubers of the same varieties planted on different dates from the beginning of April to mid-June, and all raised on the same day in October, showed no such grading in sprout length when examined at intervals in storage.

B. *Sprouting tubers.*

(i) *First sign of sprouting.* When the "eyes" became discernible as minute papillae and before the tubers would be said commercially to have begun sprouting, the effect on respiration was already to be seen in that the rate rose from 0·0037 to 0·0062 mg. with healthy tubers and from 0·0033 to 0·0057 mg. with diseased ones (Fig. 5 and Appendix II, Exp. 1). At a slightly more advanced stage of sprouting the rate increased to 0·0107 mg. for healthy and to 0·0085 mg. for diseased (Appendix II, Exp. 2). Under anaerobic conditions the rate of respiration was rather less than under aerobic (Figs. 5 and 5*a*). A similar but smaller increase was observed under anaerobic conditions as sprouting proceeded, the comparable rates with Exps. 1 and 2 above being 0·0049 and 0·0069 mg. for healthy tubers and 0·0046 and 0·0066 mg. for diseased ones respectively (Fig. 5, and Appendix VI, Exps. 2 and 4).

(ii) *Tubers with sprouts about half an inch in length.* Exps. 5–8 in Appendix II give the results obtained with tubers on which sprouts about half an inch long had developed. In all this work every care was taken to select tubers as closely comparable as possible in respect of weight and of sprout size, and this was checked by comparing healthy tubers with healthy in the paired apparatus and then following with diseased ones. Two such pairs of results are shown in Fig. 6, as well as in Appendix II. From the latter it can be seen that only in one case was there a serious discrepancy in the rate of respiration of two selected tubers, and this was due to mycelial growth on the sprouts of one tuber in Exp. 5. Deleting this aberrant result, the average respiration shown by healthy tubers with short sturdy sprouts was 0·0197 mg. and by diseased ones 0·0088 mg. Under anaerobic conditions the respective rates were 0·0097 mg. for healthy and 0·0088 mg. for diseased tubers (Appendix VI, Exp. 5).

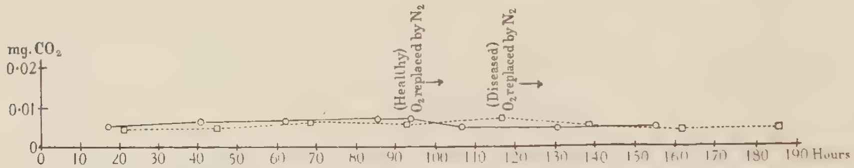

Fig. 5. Aerobic and anaerobic respiration of tubers just prior to sprouting.

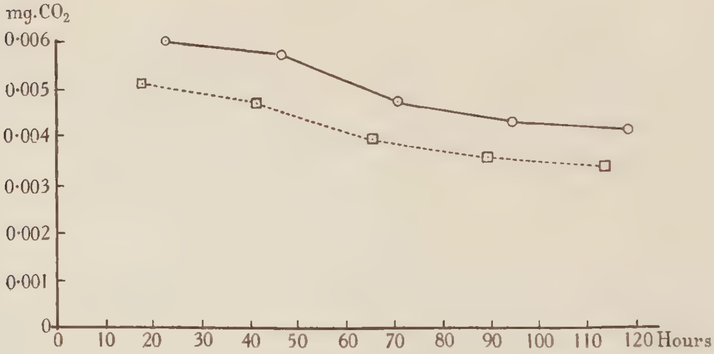

Fig. 5a. Anaerobic respiration of tubers just prior to sprouting. Healthy and leaf-roll shown as continuous and dotted lines respectively.

(iii) *Tubers with sprouts more than one inch in length.* A still further increase in respiration occurred with tubers having well-developed sprouts more than 1 in. in length, the average rates being 0·0318 mg. for healthy and 0·0262 mg. for diseased ones (Appendix II, Exps. 3 and 4). It is of particular interest that the acceleration of respiration following the renewal of growth in the tuber should be more marked with healthy tubers than with diseased ones, and that this difference should be accentuated as sprouting proceeds. It cannot directly be related to the rate of growth or vigour of the sprouts, since there is little or nothing to choose between healthy and leaf-roll tubers in this respect ((7), and unpublished work in this laboratory). On the other hand, Table I, which records the gross changes in composition of samples of ten tubers after sprouting in the dark for four and nine weeks respectively, affords a reasonable explanation of the higher respiration of healthy tubers at all stages of sprouting.

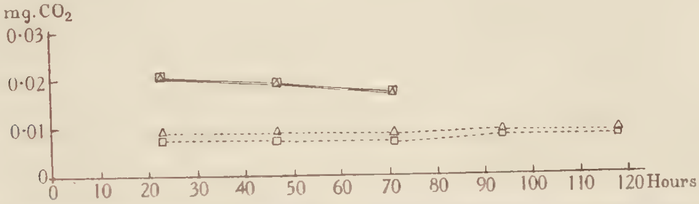

Fig. 6. Aerobic respiration of tubers with sprouts about 0·5 in. in length. Two healthy tubers (continuous lines) *or* two leaf-roll tubers (dotted lines) occupying the paired apparatus at the same time.

Table I.

Gross changes in composition of tubers and sprouts.

	% loss (H_2O + katabolism)				Change in % dry matter			
	After 4 weeks		After 9 weeks		After 4 weeks		After 9 weeks	
Material	Healthy	Leaf-roll	Healthy	Leaf-roll	Healthy	Leaf-roll	Healthy	Leaf-roll
Tubers	5·5	3·6	14·0	8·7	24·7	20·2	28·9	22·4
Sprouts	—	—	—	—	14·5	11·4	13·7	10·0

NOTE. All differences shown are mathematically significant.

C. *Covered growing plants.*

The respiration of vigorously growing plants was determined repeatedly and the results are summarised in Appendix IV for aerobic conditions and in Appendix VI for anaerobic. The two classes of plants were experimented with alternately, the foliage being enclosed in a bell-jar and the roots sealed off in the thermostat chamber kept at 20° C. The rising curve (Figs. 7 and 8) represents the stabilising period due to the increasing concentration of CO_2 in the bell-jar and could be modified by varying its capacity. It will be seen that not only is the rate of respiration of both healthy and diseased plants very much higher than in well-sprouted tubers, but that the healthy now respire at a definitely lower level than the diseased plants. The result of one such determination in plants already producing tubers (seven in the case of the healthy and two in the diseased plant) is illustrated in Fig. 7, stabilisation occurring at about 0·189 mg. for the healthy plant and at 0·276 mg. for the diseased one (cf. also Appendix IV, Exp. 2). Plants of the variety Kerr's Pink were also used and gave quite similar results. In one such test with this variety, the whole plant with its seed tuber and roots being enclosed in the thermostat respiration chamber, the curve obtained was as shown in Fig. 8. The relation between healthy and diseased is the usual one and the flatter curve is due to the inclusion of the seed tuber, which not only respired at a lower rate than the foliage but greatly increased the fresh-weight divisor to be used (cf. Appendix IV, Exp. 5). A repetition with plants known to be diseased but showing no symptoms apart from a general pallor of the foliage gave a similar result (Appendix IV, Exp. 1), so that the higher rate of respiration of diseased plants is not directly connected with the accumulation of excess starch in the leaves. Under anaerobic conditions the rate of respiration was rather higher, or the

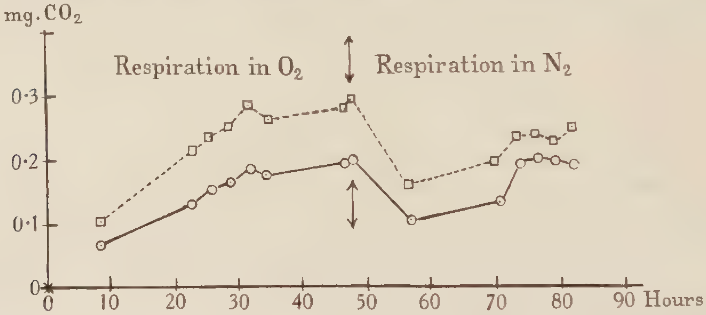

Fig. 7. **Aerobic and anaerobic respiration of growing plants; shoots only. Healthy and leaf-roll shown as continuous and dotted lines respectively.**

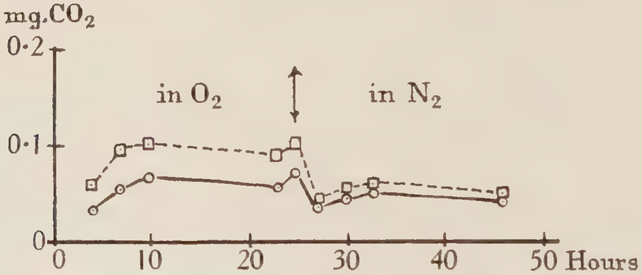

Fig. 8. Aerobic and anaerobic respiration of potato plant (Kerr's Pink), including attached seed tuber. **Healthy and leaf-roll shown as continuous and dotted lines respectively.**

same as, that under aerobic conditions; in contradistinction to the reaction of tubers, whether dormant or sprouting. The higher rate of the diseased plants as compared with the healthy was, however, unchanged except in one case where both plants had been starved of mineral food (Appendix VI, Exp. 9).

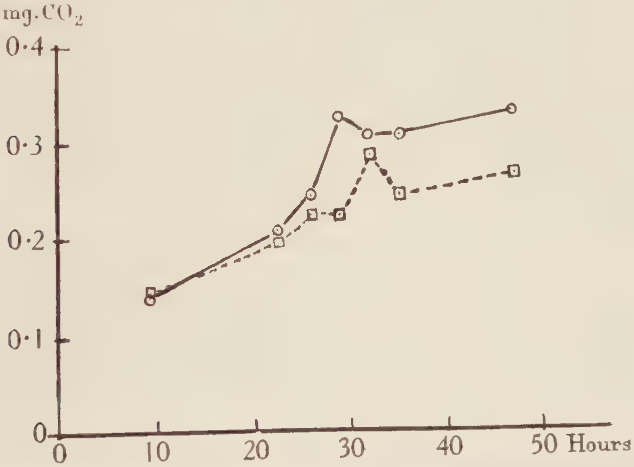

Fig. 9. Aerobic respiration of very young growing plants with first leaves just unfolded. Healthy and leaf-roll shown as continuous and dotted lines respectively.

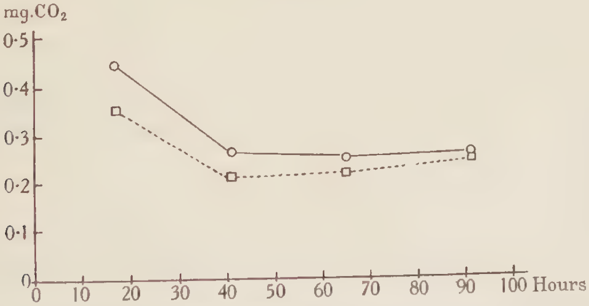

Fig. 10. Aerobic respiration of very young, cut, shoots, with petioles immersed in water. Healthy and leaf-roll shown as continuous and dotted lines respectively.

It was evident from a consideration of the results with sprouting tubers and growing plants that there must be some stage of growth at which the diseased plant overtakes the healthy one in respiratory activity, and that this stage is reached before either rolling or starch accumulation occurs in the diseased foliage. Thung's work (9) with detached leaves of different stages of maturity had indicated much the same thing and pointed to its occurring at such a young stage of development that its exact location would present much difficulty. Efforts, however, were made with plants in which the first leaves had barely expanded, the experiments including potted growing plants, very young plants lifted and the seed tuber detached, and cut leaves with the petioles standing in water or embedded in wax. In six experiments, summarised in Appendix III, two of them (Exps. 1 and 3) still show the healthy plant as having the higher respiration (cf. Figs. 9 and 10); in one case (Exp. 6) the healthy and diseased shoots respired at the same rate, and in three cases the "change over" had already occurred (Appendix III, Exps. 2, 4, and 5, and in Figs. 11 and 12). The conclusion arrived at in these experiments was that the respiration of diseased plants exceeded that of healthy plants almost as soon as the foliage unfolded from the sprouts.

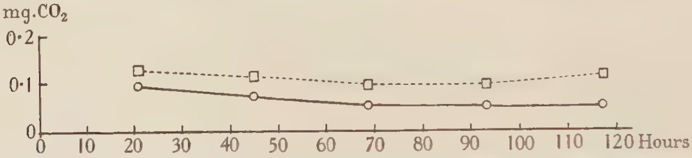

Fig. 11. Aerobic respiration of very young plant with seed tuber detached. Healthy and leaf-roll shown as continuous and dotted lines respectively.

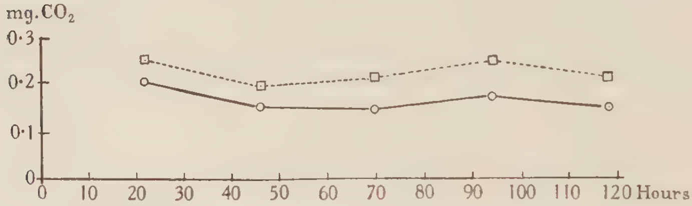

Fig. 12. Aerobic respiration of very young shoots with leaves just unfolded and petioles embedded in wax. Healthy and leaf-roll shown as continuous and dotted lines respectively.

All this work was done with plants from which light was excluded, and it seemed likely that a partial explanation of the excessive respiration of the leaf-roll plants could be found in observations made by the writer and Mr D. E. Green in 1927 and reported upon at the Leeds meeting of the British Association in that year (10). This work not only confirmed the well-known fact that little or no diminution of reserve starch occurs in diseased leaves, but also that a considerable difference exists in the rates at which reducing sugars are depleted from healthy and leaf-roll foliage during the night hours. From 1 p.m. to 5 a.m. bi-hourly estimates were made of the reducing sugars found in the laminae and petioles of both classes of plants (each estimate being made in duplicate with Fehling's solution without previous conversion of sucrose).

The conclusions reached were that at midday, healthy leaves which were full of starch were almost if not quite devoid of reducing sugars. These latter began to appear in both laminae and petioles between 4 and 6 p.m., and greatly increased during the evening hours up to 8 p.m. By that time most of the starch had been converted and there was a steep fall in the amount of reducing sugars found until by 5 a.m. both laminae and petioles were again quite clear. In the case of leaf-roll plants, however, the leaves were not only full of starch at midday but also had a large amount of reducing sugars. Depletion, *i.e.* translocation plus respiration, was very slow during the night and there was no evidence that the reserve starch in the foliage had even been drawn upon at all. As with healthy leaves, no reducing sugars were found at 5 a.m. The results, so far as depletion of starch was concerned, were confirmed by hourly determinations, throughout the night on two occasions, on leaves taken from the top, middle and base of healthy and leaf-roll plants growing in the field. Indeed, abundant starch remains in the leaves of leaf-roll plants kept covered for six days. The recent work of Barton-Wright and M'Bain(2), from which they conclude that whilst hexoses are translocated in leaf-roll plants the translocatory sugar in healthy plants is sucrose, does not affect the conclusion already drawn. There can be no doubt that the respirable substrate in healthy foliage is rapidly evacuated during a period of darkness, whilst the leaf-roll leaves retain most of the sugars, which are thus available for respiration.

D. *Experiments with growing plants periodically exposed to light.*

The obvious way of testing out the supposition that the excessive respiration of leaf-roll plants in the dark was due to the failure to translocate sugars would be to determine the respiratory activity when carbohydrate concentration in healthy and diseased plants most nearly approaches parity, *i.e.* during exposure to light. Any such attempt, however, would involve assumptions as to the relation between respiration and carbon assimilation which are not warranted by our present state of knowledge. It was therefore decided to determine the respiration, at night, of plants which had been exposed to light, and to rely on the probable greater assimilation of carbon dioxide and more rapid movement of sugars from the seed tuber in the case of the healthy plants, in order to redress the balance of carbohydrates in the foliage. The bell-jar respiratory chamber was replaced by the adjustable chamber described in Fig. 1, and the roots were freely exposed to air. The first experiments were carried out in the laboratory with the plants exposed to a north light in January. Under these conditions no assimilation at all took place, as was shown both by the maintenance of the same level of respiration whether the plants were covered or not, and by the ultimate death of established plants deprived of their seed tubers and continuously exposed to light in a roof glasshouse. Incidentally this was of interest in that potatoes are quite successfully cultivated during such low-light intensities; they appear normal in every way and produce small tubers, although the whole of the organic food must have been derived from the seed tuber.

Work was therefore postponed until the light intensity improved and preliminary tests with plants showed that some carbon assimilation was

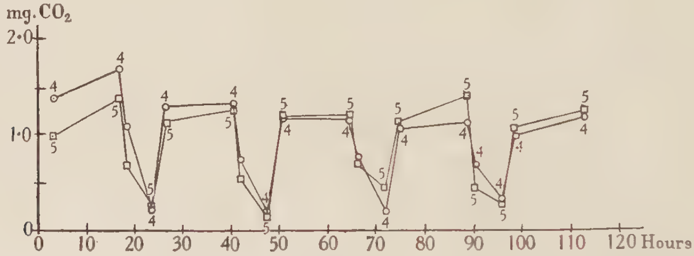

Fig. 13. Aerobic respiration, in mg. per hour per gram dry weight, of two healthy plants in the paired apparatus; the plants being periodically exposed to light.

being carried out. The reliability of the paired apparatus was also tested out by determining the respiration of two apparently comparable healthy plants. The two curves obtained are shown in Fig. 13, which gives some idea of the error to be allowed for in interpreting differences obtained when healthy and diseased plants occupied the apparatus at the same time. The month of February 1932 was exceptionally cold and considerable variation in temperature between day and night was inevitable, so that it was desirable to ascertain the effect of temperature on the respiration of healthy and leaf-roll plants before considering the main problem as to the effect on respiration of the accumulated assimilation products. Two plants from which light was excluded, one being healthy and the other diseased, occupied the apparatus for this purpose. By adjustment of the electrical and water-heating apparatus in the glasshouse the plants were exposed successively to temperatures between 35 and 85° F.; being maintained for several hours at various temperatures within this range to allow of the determination of the rate of respiration. At each determination the temperature fluctuated about 2·5° F. As a method of determining the precise effect of temperature on respiration there are obvious objections, but as a comparison between healthy and diseased plants under identical conditions the method appears to be sound enough. The temperature-respiration curves are shown as heavy continuous lines in Figs. 14 and 15 for healthy and leaf-roll plants respectively. The curve of nearest fit was found by the formula $T=x+by+cz$, in which x, y, and z are the three variable coefficients to be determined; T is the temperature in ° F.; b = the milligrams of CO_2 evolved and $c=b^2$. The curve for the diseased plant (which showed heavy starch accumulation both before and after the experiment) is similar in form to that for the healthy plant but is steeper and at a much higher level throughout. It will be observed that the correlation between temperature and respiration is much closer in the healthy than in the diseased plant.

(i) *Plants exposed to low light intensity and supplied with* CO_2*-free air.* Three pairs of plants were tested under low light intensities which were insufficient for the plants to make good the loss of respiratory CO_2. The differences observed between the rate of respiration when exposed and when not exposed to light indicated that the healthy plants were much more efficient in utilising such light and CO_2 as were available, and it seemed therefore a reasonable assumption that the disparity between healthy and diseased foliage in regard to carbohydrate content was partially rectified. In the first two pairs the diseased plants showed only the pallid wavy foliage characteristic of leaf-roll plants under glass in winter, and basal leaves detached for the purpose had only traces of excess starch present. The diseased plant used in the third experiment, however, had all the typical leaf-roll symptoms including heavy accumulation of starch in the basal leaf after standing in water in the dark for 48 hours. The results are summarised in Appendix V, and curves illustrating the first and third experiment are given in Figs. 16 and 17. It is evident that there is little difference in the rate of respiration of the first two pairs of plants, whereas the third diseased plant which had accumulated a large amount of excess starch had a much higher rate than the comparable healthy plant. The figures and summary in Appendix V are given on a dry-weight basis, but it is equally true when fresh weight is taken, the figures being 0·13 and 0·11 mg. for healthy and diseased respectively in the first pair of plants, 0·095 mg. for both in the second pair, whilst for the third pair the means were 0·0883 and 0·1342 mg. for

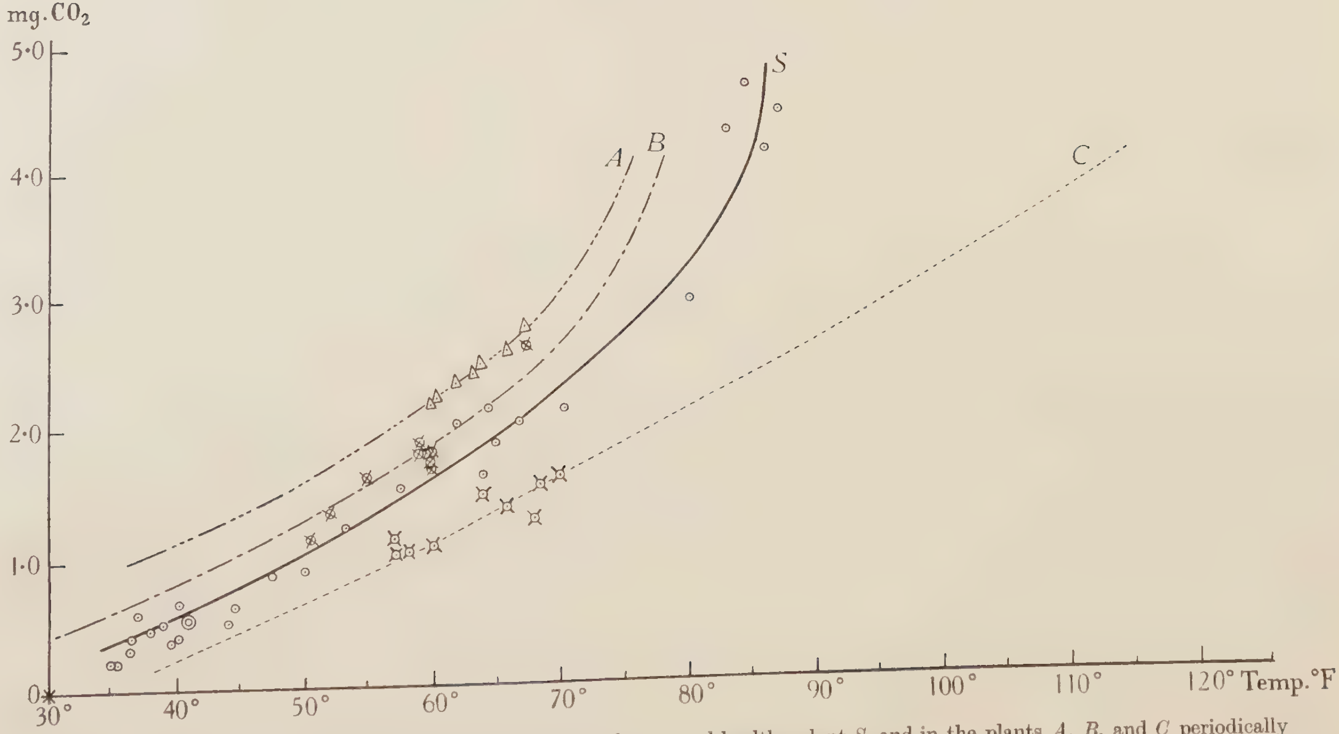

Fig. 14. Relation of aerobic respiration to temperature in the covered healthy plant *S*, and in the plants *A*, *B*, and *C* periodically exposed to light. *A*, *B*, *S*, and *C* represent progressively older healthy plants.

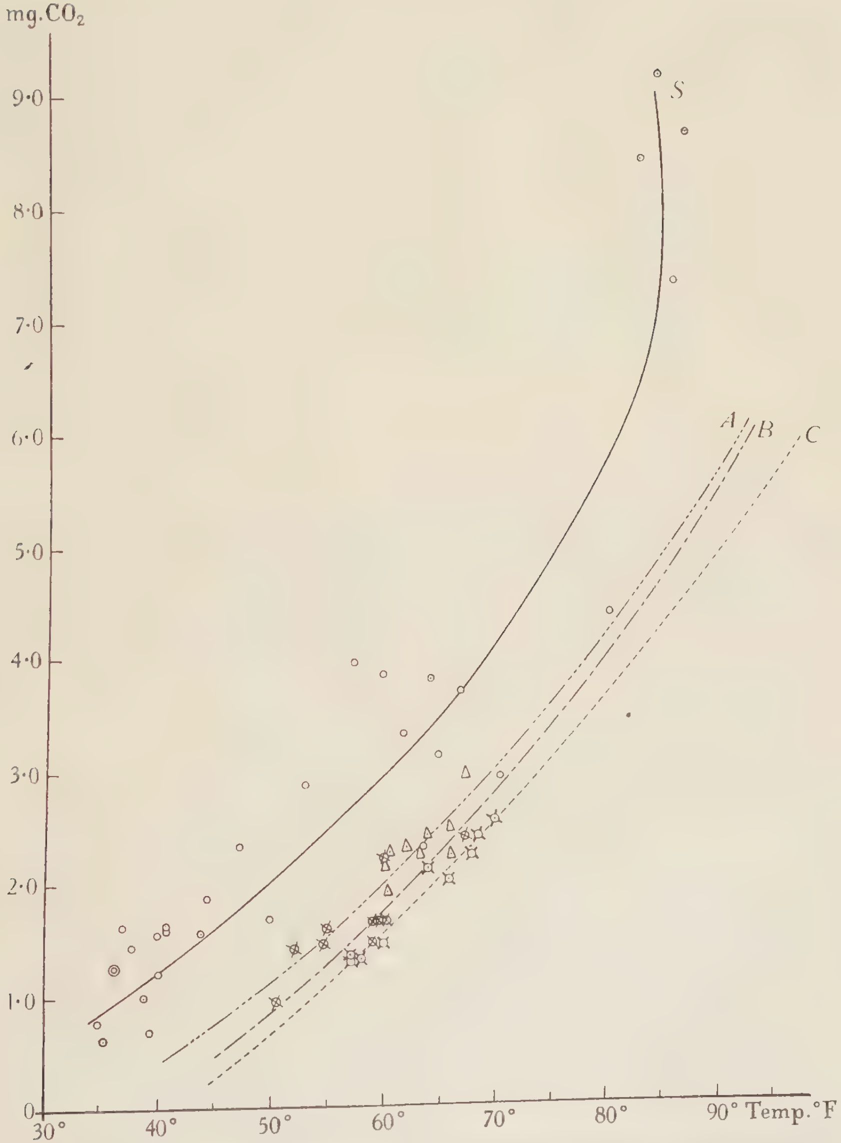

Fig. 15. Relation of aerobic respiration to temperature in the covered leaf-roll plant *S*, and in diseased plants *A*, *B*, and *C* periodically exposed to light. *A*, *B*, *S*, and *C* represent progressively older plants.

healthy and diseased respectively. During these experiments the temperature varied between one night and the next over a range of about 15° F., both classes of plants being exposed, of course, to the same vicissitudes of temperature. The rates of respiration obtained at the

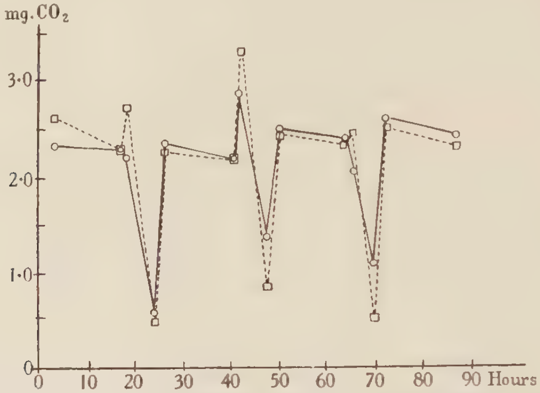

Fig. 16. Aerobic respiration of healthy and leaf-roll plants exposed to low light intensity and supplied with CO_2 air. No foliage rolling and practically no excess starch accumulation in diseased plant. Healthy and diseased shown as continuous and dotted lines respectively. Respiration in mg. per hour per gram dry weight.

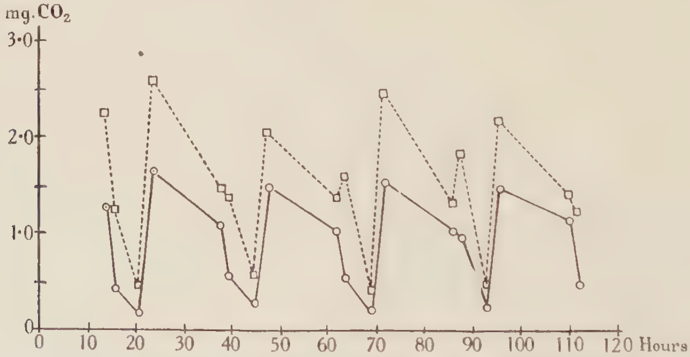

Fig. 17. Aerobic respiration of healthy and leaf-roll plants as in Fig. 16, but diseased plant showed definite foliage rolling and heavy excess starch accumulation. Healthy and diseased shown as continuous and dotted lines respectively. Respiration in mg. per hour per gram dry weight.

various temperatures within this small range were plotted for each plant for comparison with each other and with the standard plant *S*. In Fig. 14, which gives the reaction of healthy plants, the curves *A*, *B*, *S*, and *C* refer to progressively older plants, and it will be noted that they fall into a series of decreasing rates of respiration. The same is true of the diseased plants shown in Fig. 15, so far as *A*, *B*, and *C* are concerned, although the correlation with age is much less evident than in the case of the healthy plants. The diseased plant *S*, however, which in point of age falls between *B* and *C*, showed an abnormally high rate of respiration which cannot be attributed to anything other than the large accumulation of starch shown by this plant.

(ii) *Plants exposed to light of high intensity and supplied with atmospheric carbon dioxide.* The last three experiments differed conspicuously from normal field conditions in the low light intensity and in the fact that no addition to the total carbohydrate content of the plants was possible. The healthy plants were found to have a definitely higher power of assimilating carbon dioxide than the diseased plant, although the latter always had the advantage of a much larger amount of respiratory carbon dioxide available for assimilation. Neither class of plant was, however, able to utilise more than a fraction of the respiratory carbon dioxide under these low light intensities, so the work was postponed for another month until the light intensity (as measured by the rate at which iodine was released from solution when acted upon by sulphuric acid) had more than doubled, and was in fact equal to that recorded in August in the English Lake district (8). Four experiments were carried out in which atmospheric air was admitted both to the two respiratory chambers and to a third control Pettenkofer tube; the respiration being found by subtraction. This continuous control was essential because the concentration of atmospheric carbon dioxide varied considerably during

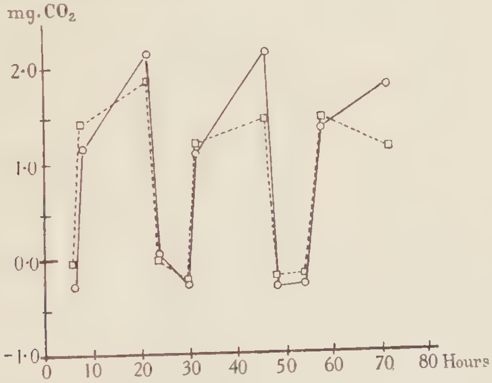

Fig. 18. Aerobic respiration of healthy and leaf-roll plants exposed to high light intensity and supplied with atmospheric carbon dioxide. Diseased plant showing little excess starch accumulation. Healthy and diseased shown as continuous and dotted lines respectively. Respiration in mg. per hour per gram dry weight.

intermittent sunshine and rain, or between day and night. Only one diseased plant was tested before any appreciable amount of excess starch had accumulated in the leaves; the mean rate of respiration being in favour of the healthy plant (Fig. 18 and Appendix V, Exp. 5). In the remaining three cases, in which the diseased plants had abundant starch already present in the leaves, the healthy plants were only able to respire at a much lower rate than the diseased plants, notwithstanding the increased opportunity to approach parity in carbohydrate content afforded by the high light intensity (Fig. 19).

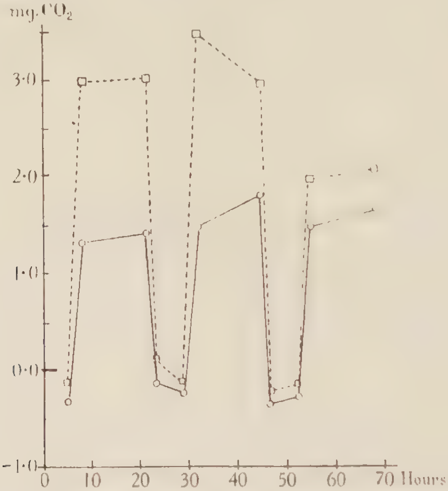

Fig. 19. As in Fig. 18, but diseased plant showed definite foliage rolling and heavy excess starch accumulation. Healthy and diseased shown as continuous and dotted lines respectively. Respiration in mg. per hour per gram dry weight.

We may safely conclude that leaf-roll plants show the higher rate of respiration not only when light is excluded but also when the two classes of plants are exposed to high light intensities—for hexoses are more abundant in diseased foliage than in healthy during daylight under normal field conditions (10), and the higher temperature of the day would still further increase the respiration of diseased plants as compared with healthy ones (cf. Figs. 14 and 15).

IV. Discussion.

The facts ascertained in this work, and serving as a basis for discussion, are brought together in Fig. 20. From *A* to *E* represents the results obtained with tubers and growing plants, *i.e.* the whole life cycle of the plant. The respiration of plants exposed to low light intensities and dependent solely upon the respiratory carbon dioxide for assimilatory purposes, with therefore no possibility of increasing the initial amount

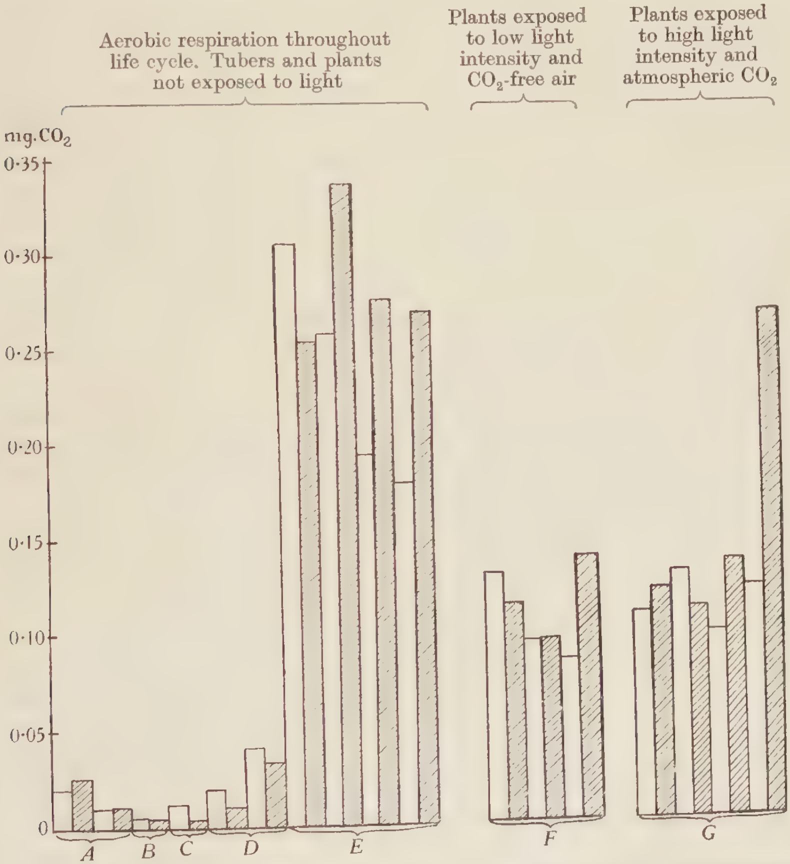

Fig. 20. Block graph summarising the work on the aerobic respiration of healthy and leaf-roll potatoes. *A*, immature tubers immediately on lifting and again after storage. *B*, stable rate in the later stages of storage. *C* and *D*, successive stages in sprouting. *E*, rate of respiration in progressively older plants from left to right. *F*, plants exposed to low light intensity and supplied with CO_2-free air. *G*, plants exposed to high light intensity and supplied with atmospheric CO_2. In each experiment the healthy material is shown on the left of the leaf-roll counterpart; the latter being shaded.

of carbohydrates present, is given in *F*, whilst *G* represents as nearly as possible the normal field conditions in that the plants were exposed to high light intensities and were able to utilise atmospheric carbon dioxide in addition to the product of respiration.

During the whole life of the plant there is only a short period, covering the end of dormancy to the first unfolding of the leaves, when the healthy plant respires at a higher rate than that of the diseased plant. At all other times, whether at the beginning of dormancy or throughout almost the whole period above ground, the diseased plant respires at much the higher rate. There is no evidence from this work that the presence of the virus has any direct influence on the rate of respiration, but only in so far as it affects the amount of available substrate required for respiration. The high rate of respiration of immature healthy tubers as compared with fully mature ones is said to be correlated with a difference in the sucrose-hexose ratio at the two stages (1), and it would be useful to determine whether this is substantiated by an analysis of leaf-roll tubers. In any case the fall during this period is continuous and there is evidently no breakdown in "organisation resistance" such as is postulated by Blackman to account for the rise in respiration found with fruit during the earlier stages of maturation. The retardation in translocation of food from the diseased seed tuber is plainly as largely responsible for the relatively low respiration of the diseased sprouting tuber as it is for the high rate of respiration of the diseased foliage, though it seems probable that here, also, the nature of the predominant sugar is of importance. This excessive respiration of the leaf-roll growing plant has no very obvious value, for the plant remains stunted, with shortened internodes and rather smaller leaves than normal plants, while the "misuse" of the leaves as carbohydrate storage organs prevents the proper development of tubers. If the expression may be allowed, everything points to respiration being "involuntary" and a necessary concomitant of the presence of certain forms of substrate; the energy released in the respiration of the leaf-roll infected plant is uneconomic and largely wasted.

V. SUMMARY.

1. A comparative study of the rates of respiration, as measured by the weight of carbon dioxide evolved, has been made with healthy and leaf-roll infected potatoes at all stages in the life cycle, under anaerobic as well as aerobic conditions.

2. Except for a short period covering the end of dormancy of the tuber to the first unfolding of the leaves, the infected plant respires at a much higher rate than does the healthy one. This is true also when the conditions of light, temperature, and external carbon dioxide approximate to those present in the field.

3. The rate of respiration is not directly related to the presence of the virus but rather to the available amount of respirable substrate. Normally the accumulation of such substances in the leaves of leaf-roll plants occurs at a very early stage of development, but can be delayed by continuous exposure to light of low intensities. Under these latter conditions the rate of respiration of diseased plants approximates to that of healthy ones.

VI. ACKNOWLEDGMENT.

The writer wishes to express his indebtedness to the laboratory assistant, Mr G. L. Turner, for valuable and unstinted help during the present work.

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APPENDIX I.

Mean aerobic respiration of immature and mature dormant tubers.

No. Exp.	Condition of tubers	Type of tubers	Fresh weight (gm.)	No. of readings	Total period (hours)	Temp. ° C.	mg. CO_2 per gm. per hr. fresh weight	
							A	*B*
1	Immature; July lifted. Kept on moist sand for three days	Healthy	37·89	5	70	18·1	0·0487	0·0279
		Leaf-roll	39·21	5	70	18·1	0·0544	0·0292
2	As above (1) but peeled	Healthy	35·75	9	135·5	19·6	0·0321	0·0226
		Leaf-roll	37·28	9	135·5	19·6	0·0429	0·0283
3	Immature; August lifted. Used at once	Healthy	78·82	7	88·25	17·6	0·0180	0·0180
		Leaf-roll	94·71	7	88·25	17·6	0·0275	0·0275
4	Same tubers as (3) after one month's storage	Healthy	75·98	6	141·75	17·8	0·0080	0·0066
		Leaf-roll	90·81	6	141·75	17·8	0·0107	0·0087
5	Same tubers as (3) after three months' storage	Healthy	73·47	5	119·25	17·0	0·0038	0·0038
		Leaf-roll	88·03	5	119·25	17·0	0·0046	0·0046
6	Immature; August lifted. After three weeks' storage	Healthy	84·03	11	264·00	14·3	0·0095	0·0092
		Leaf-roll	90·80	11	264·00	14·3	0·0087	0·0086
7	Same tubers as (6) one week later	Healthy	83·45	6	143·50	15·0	0·0068	0·0068
		Leaf-roll	90·28	6	143·50	15·0	0·0069	0·0069
8	Mature; September lifted. Used at once	Healthy	76·73	4	95·0	17·5	0·0074	0·0074
		Leaf-roll	71·90	4	95·0	17·5	0·0094	0·0094
9	Mature; September lifted. After five weeks' storage	Healthy	514·0	5	119·25	20·75	0·0066	0·0066
		Leaf-roll	490·0	5	119·25	18·25	0·0077	0·0077
10	Mature; October lifted. After five months' storage	Healthy	600·0	3	52·00	20·00	0·0037	0·0037
		Leaf-roll	600·0	3	52·00	20·00	0·0033	0·0033

A = Mean respiration over whole period. *B* = Mean respiration after stabilising.

APPENDIX II.

Mean aerobic respiration of sprouting tubers.

No. Exp.	Condition of tubers	Type of tubers	Fresh weight (gm.)	No. of readings	Total period (hours)	Temp. ° C.	mg. CO_2 per gm. per hr. fresh weight after stabilising
1	October lifted. Used just prior to sprouting	Healthy	590·0	5	95·25	19·5	0·0062
		Leaf-roll	579·0	5	117·5	19·5	0·0057
2	October lifted. Sprouts less than 0·25 in. long	Healthy	625·0	3	30·0	19·5	0·0107
		Leaf-roll	624·0	5	24·0	19·5	0·0085
3	As for (1) but sprouted on wet sand for one month. Sprouts 1 in. long	Healthy	590·0	3	18·0	19·5	0·0254
		Leaf-roll	579·0	3	18·0	19·5	0·0210
4	As for (3) but one week later	Healthy	590·0	5	24·0	19·5	0·0383
		Leaf-roll	579·0	5	21·75	19·5	0·0314
5	Single tubers sprouted slowly on bench	Healthy	39·56	4	95·0	19·25	0·0181
		Healthy	31·98	4	95·0	19·25	0·0239*
6	As for (5)	Leaf-roll	27·91	5	118·0	18·25	0·0094
		Leaf-roll	31·26	5	118·0	18·25	0·0092
7	As for (5)	Healthy	32·68	3	72·0	19·5	0·0191
		Healthy	32·91	3	72·0	19·5	0·0190
8	As for (5)	Leaf-roll	50·93	5	118·0	18·9	0·0077
		Leaf-roll	36·26	5	118·0	18·9	0·0090

* Fungal mycelium developed on one sprout.

APPENDIX III.

Mean aerobic respiration of plants with leaves just unfolding.

No. Exp.	Condition of plants	Type of plant	Fresh weight (gm.)	No. of readings	Total period (hours)	Temp. ° C.	mg. CO_2 per gm. per hr. fresh weight
1	10 in. high; growing in pots	Healthy	11·0	5	25·0	19·2	0·3069
		Leaf-roll	12·5	5	24·0	19·2	0·2544
2	4 in. high; lifted, washed and mother tuber detached	Healthy	15·5	5	117·0	15·8	0·0664
		Leaf-roll	5·0	5	117·0	15·8	0·1114
3	3 in. high; cut and ends placed in water	Healthy	2·24	4	90·0	16·8	0·2613
		Leaf-roll	1·015	4	90·0	16·8	0·2336
4	As for (3) but cut ends sealed with paraffin wax	Healthy	1·61	5	118·75	17·1	0·2211
		Leaf-roll	1·22	5	118·75	17·1	0·2801
5	As for (3) but cut ends sealed with grafting wax	Healthy	1·105	5	117·75	19·9	0·1645
		Leaf-roll	1·605	5	117·75	19·9	0·2204
6	As for (3)	Healthy	0·555	5	117·25	15·8	0·1976
		Leaf-roll	0·462	5	117·25	15·8	0·2028

APPENDIX IV.

Mean aerobic respiration of covered growing plants.

No. Exp.	Condition of plants	Type of plant	Fresh weight (gm.)	No. of readings	Total period (hours)	Temp. ° C.	mg. CO_2 per gm. per hr. fresh weight	
							A	*B*
1	7–9 in. high; 39 leaves. L.R. pale but not rolled	Healthy	46·5	8	47·25	18·7	0·2360	0·2610
		Leaf-roll	27·5	8	47·25	18·7	0·3080	0·3510
2	9 in. high; 53 leaves. L.R. definitely rolled	Healthy	44·5	8	47·75	19·0	0·1320	0·1890
		Leaf-roll	23·5	8	48·0	19·3	0·2390	0·2760
3	10 in. high; healthy 47 leaves; L.R. 20 leaves and definitely rolled	Healthy	42·0	8	47·25	19·9	0·1561	0·2116
		Leaf-roll	26·0	8	48·0	18·7	0·2407	0·2778
4	Growing in silver sand without artificial manure, pale, silvery leaves	Healthy	38·5	8	47·75	18·2	0·1832	0·2125
		Leaf-roll	33·5	8	48·0	19·1	0·1926	0·2250
5	Kerr's Pink, lifted with tuber and roots intact	Healthy	145·0	5	25·5	19·5	0·0542	0·0603
		Leaf-roll	120·0	5	25·5	19·5	0·0874	0·0949
6	Kerr's Pink, growing in pots	Healthy	25·0	1	22·0	18·5	0·1034	0·1034
		Leaf-roll	20·0	1	22·0	19·5	0·1486	0·1486
7	Kerr's Pink, growing in pots	Healthy	47·0	8	46·75	19·0	0·1458	0·1651
		Leaf-roll	44·0	8	47·0	19·7	0·2065	0·2360

A = Mean respiration over whole period. *B* = Mean respiration after stabilising.

APPENDIX V.

Mean aerobic respiration of growing plants periodically exposed to light.

Experimental conditions	No. Exp.	Type	Starch reaction	Respiration during successive nights (mg. CO_2 per hr. per gm. dry weight)										Mean respiration	Mean temp. ° F.	Mean light intensity (c.c. I_2)
				(1)	Temp. ° F.	(2)	Temp. ° F.	(3)	Temp. ° F.	(4)	Temp. ° F.	(5)	Temp. ° F.			
Low light intensity. CO_2-free air supplied	1	H. 21 *a*	−	2·2945	62·75	2·2506	63·0	2·4088	63·0	2·5013	64·75	—	—	2·3638	63·5	1·08
		L.R. 16 *b*	±	2·4432	62·75	2·2117	63·0	2·3703	63·0	2·3878	64·75	—	—	2·3533	63·5	1·08
	2	H. 16 *a*	−	2·2013	63·5	1·9023	56·75	1·4636	55·25	1·6615	57·5	1·6076	55·5	1·7673	57·7	1·51
		L.R. 16 *a*	$\underline{\pm}$	2·0550	63·5	1·4727	56·75	1·3152	55·25	1·9158	57·5	1·5462	55·5	1·6650	57·7	1·51
	3	H. 15 *a*	−	—	—	1·3593	65·0	1·2044	61·5	1·2953	63·25	1·2981	60·5	1·2893	62·75	2·37
		L.R. 18 *a*	+ ±	—	—	2·0186	65·0	1·6970	61·5	1·8760	63·25	1·7715	60·5	1·8408	62·75	2·37
High light intensity. Atmospheric CO_2 supplied	4	H. 23 *a*	−	1·8119	67·0	0·9107	60·25	0·9190	59·25	—	—	—	—	1·2139	62·5	3·17
		L.R. 24 *a*	+ ±	2·8587	67·0	1·3081	60·25	0·8783	59·25	—	—	—	—	1·6817	62·5	3·17
	5	H. 23 *b*	−	1·6631	58·5	1·6466	58·75	1·6102	58·0	—	—	—	—	1·6406	58·5	3·23
		L.R. 24 *b*	±	1·6678	58·5	1·3554	58·75	1·3481	58·0	—	—	—	—	1·4571	58·5	3·23
	6	H. 24 *b*	−	1·3569	63·5	0·9604	59·5	1·1572	62·75	—	—	—	—	1·1582	62·0	3·87
		L.R. 23 *a*	+ +	1·4251	63·5	1·3531	59·5	1·3442	62·75	—	—	—	—	1·3742	62·0	3·87
	7	H. 24 *a*	−	1·3636	72·0	1·6417	71·75	1·5545	65·75	—	—	—	—	1·5199	69·83	3·97
		L.R. 23 *b*	+ ±	2·9953	72·0	3·1989	71·75	1·9960	65·75	—	—	—	—	2·7301	69·83	3·97

H. = healthy; L.R. = leaf-roll.

+ +; + ±; ±; $\underline{\pm}$; − = successively decreasing starch reaction.

APPENDIX VI.

Mean respiration in nitrogen of plants at all stages of life cycle.

No. Exp.	Condition of tuber or plant	Type of tuber or plant	Fresh weight (gm.)	No. of readings	Total period (hours)	Temp. ° C.	mg. CO_2 per gm. per hr. fresh weight	
							A	*B*
1	Mature tubers after three weeks' storage	Healthy	494·5	5	118·5	17·5	0·0061	0·0054
		Leaf-roll	430·0	5	118·5	18·0	0·0068	0·0061
2	Mature tubers just prior to sprouting	Healthy	590·0	3	61·0	19·5	0·0049	0·0049
		Leaf-roll	579·0	3	69·5	19·5	0·0046	0·0046
3	As for (2)	Healthy	237·0	5	119·25	17·2	0·0050	0·0044
		Leaf-roll	232·0	5	114·5	16·4	0·0041	0·0036
4	Tubers with sprouts less than 0·25 in. long	Healthy	616·0	6	61·25	19·5	0·0069	0·0069
		Leaf-roll	617·0	6	67·25	19·5	0·0066	0·0066
5	10 tubers sprouted slowly on bench	Healthy	378·0	9	45·0	19·5	0·0097	0·0097
		Leaf-roll	379·0	10	47·5	20·0	0·0088	0·0088
6	Growing plants, cf. Appendix IV, Exp. 2	Healthy	44·5	6	34·5	18·2	0·1720	0·1960
		Leaf-roll	23·5	6	34·5	18·6	0·2130	0·2320
7	Growing plants, cf. Appendix IV, Exp. 3	Healthy	42·0	6	34·5	20·0	0·2007	0·2321
		Leaf-roll	26·0	6	35·0	19·2	0·2433	0·2862
8	Kerr's Pink, growing plants, cf. Appendix IV, Exp. 5	Healthy	145·0	4	21·5	19·5	0·0412	0·0434
		Leaf-roll	120·0	4	21·5	19·5	0·0438	0·0457
9	Growing in silver sand without artificial manure, cf. Appendix IV, Exp. 4	Healthy	35·5	6	33·0	17·6	0·2386	0·2773
		Leaf-roll	33·5	6	33·5	19·9	0·1971	0·2311
10	Growing plants, L.R. winter symptoms, wavy margins, no starch accumulation	Healthy	31·0	4	93·0	18·75	0·1145	0·1283
		Leaf-roll	17·0	4	95·0	19·75	0·1508	0·1639
11	Growing plants, as for (10) but L.R. starch accumulation	Healthy	40·5	4	94·5	18·5	0·0761	0·0814
		Leaf-roll	17·5	4	95·0	18·0	0·1922	0·2160

A = Mean respiration over whole period. *B* = Mean respiration after stabilising.

(*Received May* 15*th*, 1933.)

THE EFFECT OF MOSAIC DISEASE UPON CERTAIN METABOLIC PRODUCTS IN THE TOBACCO PLANT

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There is now evidence that virus diseases may cause decided alterations in the composition of plant tissues which are infected. An examination of previous work shows that a number of effects have been recorded for different virus diseases, and that these usually centre round alterations in the relative proportions of nitrogenous and carbohydrate constituents. It is already evident that differences exist between the effects of virus diseases on different plants. Thus, while potato leaves infected with leaf-roll have subnormal proportions of nitrogen (3), in tobacco leaves, as will be shown, the proportions of nitrogen are higher in infected plants and carbohydrates lower. Much of the previous data on this subject, however, shows evidence of large sampling errors. In determining the effect of mosaic disease upon tobacco leaves, attention was therefore paid to this point. Analyses were made to determine the effect of the disease upon the principal constituents of the leaves. On the basis of the results of these analyses, an attempt was made to confirm the indications as to the metabolic effects of the virus.

Material.

Plants of *Nicotiana tabacum* were grown in greenhouses specially constructed to preclude the entry of insects.

For each set of experiments forty healthy seedlings were selected. They were grown in the same house so that each plant was under similar conditions of light, humidity and temperature. Of these thirty plants were inoculated by scratching with a sterilised needle dipped in inoculum freshly prepared by grinding in a sterilised mortar leaves of *N. tabacum* showing mosaic mottling. These plants provided the diseased material. The remaining ten were similarly scratched, but the needle carried no inoculum. This was a control on the possible effects of scratching. These plants provided the healthy material.

About 3 weeks after inoculation, each plant was repotted in a 12 in. pot, and left to grow for another 3 weeks. By that time the leaves were considered ready for gathering, as the light and dark green areas were then fairly distinct so that pieces of each could be cut out. Considerable care was given to the selection of the leaves. Counting from the growing point, leaves nos. 4, 5, 6 and 7 were gathered from each plant. These were approximately of the same age and were fairly uniform in size. Table I gives data obtained by the measurement of twenty healthy and twenty diseased leaves.

Table I.

Leaves	Average length in.	Greatest length in.	Smallest length in.
Healthy	10·3	11	9·75
Diseased	8·7	9·5	8·5

PREPARATION OF MATERIAL.

The leaves were lightly brushed to remove dust and any superfluous moisture, and then the mid-ribs were cut out and discarded.

The material was preserved for analysis by drying according to the method suggested by Link and Tottingham (9). This method has been shown to be satisfactory for the study of carbohydrate metabolism by Link and Tottingham, and for the study of nitrogen metabolism by Pearsall and Wright (11). The leaves were well spread out, lower side uppermost, in a large oven, heated for half an hour at 95° C. to destroy enzymes and then to constant weight at 60° C. for 2 days. They showed no discoloration after drying.

The most suitable method of extraction seemed to be that suggested by Osborne, Wakeman and Leavenworth (10), and by Vickery (11), as this extraction separates the soluble and insoluble compounds of nitrogen as well as the soluble and insoluble carbohydrates. The leaves, after being finely powdered, were therefore covered with 60 per cent. alcohol (100 c.c. to 1 gm. of dry matter), and left for 24 hours. The solution was then filtered through a Buchner funnel, and the residue and filter well washed with more 60 per cent. alcohol until the washings appeared only faintly green. The residue was dried for 6 hours at 60° C. and then ground to a fine powder.

METHODS.

It is generally assumed that the change from insoluble to soluble nitrogen is represented by a reversible reaction, the insoluble nitrogen, protein in the main, being transformed into amino acids, which are then oxidised to give ammonia and probably other soluble forms of nitrogen:

$$\text{Protein} \rightarrow \text{amino acids} \rightarrow \text{ammonia} \rightarrow \text{other soluble forms (Rest N).}$$

On this assumption is based the nitrogen analysis which obtains figures for protein N, amino N, ammonia N and "other soluble forms," which for convenience is referred to as Rest N.

Protein N and total soluble N were determined by the Kjeldahl-Gunning method for micro quantities, the method modified to include nitrates being used for the determination of the soluble N. Amino N was estimated by Sorensen's formol titration, which measures (ammonia N + amino N). This method was chosen because the titration could be run in conjunction with titratable acidity determinations. The end-point was determined by a comparator method modified from that of Cole (4). In order to obtain the figure for amino N it was necessary to measure ammonia N. This was done by Van Slyke's method of aeration by suction. Actually the amount of ammonia in the first set of leaves was so small that it was disregarded, and therefore no measurement was made in the second set. This neglect of ammonia is quite a common experimental procedure, as it has been shown that, in most non-acid plants, the amount of ammonia present is very small and the range over which it varies negligible. The figures for Rest N are the differences between soluble N and amino N.

Carbohydrate estimations were carried out at first (Tables II–IV) by the picrate method of Willaman and Davison (14). This method is rapid and reliable for comparative results. The subsequent sugar estimations employed the method of Hagedorn and Jensen (8). The insoluble carbohydrates were found, in preliminary determinations, to be completely hydrolysed by 3 per cent. sulphuric acid at 100° C. for 3 hours. This procedure was subsequently employed.

The results are given as percentages of the dry weight. Estimations of titratable acidity and some of the hydrogen-ion concentration of the expressed sap are not given as, though determined, they showed no significant difference between healthy and diseased leaves.

Table II.

	Protein N	Amino N	Rest N	Total sugars	Insoluble carbohydrate
Healthy 1	3·32	0·133	0·303	9·24	32·07
2	3·41	0·141	0·304	9·02	31·75
Difference*	2·68	5·840	0·330	2·04	1·00
Diseased 1	3·80	0·176	0·293	7·57	26·99
2	3·82	0·174	0·296	7·56	27·50
Difference*	0·44	1·140	1·210	2·47	1·87

* As percentage of the mean value.

The agreement between the results for duplicate samples may be taken as indicating the magnitude of the sampling and analytical errors.

The above figures represent samples of twenty leaves each, collected in June, the data being given as percentages of the dry weight.

It will be seen that the sampling and analytical errors are reasonably small, in general, less than 3 per cent. of the mean.

RESULTS.

The results discussed here were obtained from material gathered in June, August and September. Only one sample of the June material is considered, since both samples have been shown to be similar.

Table III gives the figures for the various carbohydrate fractions.

Table III.

	As glucose				
	Hexoses	Disaccharides	Total sugars	Insoluble carbohydrate	Total carbohydrate
		June material.			
Healthy	4·712	4·338	9·050	31·753	40·803
Diseased	4·364	3·395	7·759	27·500	35·259
		August material.			
Healthy	7·844	1·165	9·009	16·062	25·071
Diseased	5·761	1·052	6·813	12·501	19·314
		September material.			
Healthy	4·271	2·322	6·593	37·01	43·60
Diseased	3·175	1·360	4·535	33·72	38·25

All figures as percentages of dry weight.

The table shows clearly that the total carbohydrate content of diseased leaves is less than that of healthy leaves and, further, that this difference is much outside any sampling error. It also shows that diseased leaves in comparison with healthy leaves contain less of all forms of carbohydrates. No light, however, is thrown by this table on the relations between the forms of carbohydrate, as the total carbohydrate content of diseased and healthy leaves is different in amount. In this respect a convenient method of expression is to calculate each form as a percentage of the total carbohydrate.

Table IV.

	Hexoses	Disaccharides	Insoluble carbohydrate	Total carbohydrate
		June material.		
Healthy	11·6	10·6	77·8	100
Diseased	12·4	9·6	78·0	100
		August material.		
Healthy	31·3	4·7	64·0	100
Diseased	29·8	5·5	64·7	100
		September material.		
Healthy	9·8	5·3	84·9	100
Diseased	8·3	3·5	88·2	100

It then becomes obvious that the proportions of hexoses, non-reducing sugars and insoluble carbohydrates in diseased leaves are not appreciably different from the proportions in healthy leaves, which suggests that the equilibria between the various forms of carbohydrate are similar in the two kinds of leaves. It therefore follows that the small amount of each of these forms in diseased leaves is not due to any disturbance of the carbohydrate balance. Other possible explanations are decreased photosynthetic activity and/or increased respiratory activity. Further analytical data is required before these can be evaluated separately, but in view of Dunlap's (5) evidence for young leaves, it appears possible that the explanation is increased respiration. The low values for disaccharides in the oldest leaves (September) and the higher insoluble carbohydrate in these leaves should be compared with the results of the subsequent respiration experiments.

Table V gives the figures obtained from the nitrogen analyses.

Table V.

	Protein N	Amino N	Rest N	Total N
	June material.			
Healthy	3·410	0·141	0·304	3·854
Diseased	3·818	0·174	0·296	4·288
	August material.			
Healthy	3·109	0·182	0·355	3·646
Diseased	3·883	0·238	0·354	4·475
	September material.			
Healthy	2·003	0·096	0·190	2·289
Diseased	2·387	0·123	0·200	2·710

All figures as percentages of dry weight.

These figures show that, compared with healthy leaves, diseased leaves contain higher total N, protein N and amino N, but a similar amount of Rest N which indicates some disturbance of the nitrogen transformations in the oxidation change between the amino acids and the Rest N. In regard to this the figures expressed as a percentage of the total nitrogen are illuminating.

Table VI.

	Protein N	Amino N	Rest N	Total N
	June material.			
Healthy	88·43	3·67	7·90	100
Diseased	89·02	4·06	6·92	100
	August material.			
Healthy	85·29	4·97	9·76	100
Diseased	86·78	5·31	7·91	100
	September material.			
Healthy	87·51	4·21	8·28	100
Diseased	88·04	4·55	7·41	100

In each series of leaves the proportion of protein is slightly higher in the diseased leaves. On the other hand the diseased leaves have lower Rest N, and this is accompanied by accumulation of amino N which suggests that the virus leads to less active oxidation of amino acids.

Attempts were also made to compare the yellow and green areas in diseased leaves. The results indicate that, while the whole of the infected leaves should be regarded as diseased, judging by their chemical composition, the yellow areas show more pronounced effects than the green ones. As the results show differences in composition between yellow and green areas of the order of those already recorded for healthy and diseased leaves, they are not given here, because there is obviously a much larger sampling error in cutting out areas from the same leaves.

These analyses, therefore, show that nitrogenous constituents are more abundant in diseased leaves than are carbohydrates. They indicate that amino-acid oxidation and protein breakdown are relatively retarded in diseased leaves, and the lower carbohydrate content might be interpreted to mean that carbohydrate oxidation is relatively more rapid. Moreover, the scarcity of disaccharides in the oldest diseased leaves suggests that some form of carbohydrate loss is more rapid in these leaves. The principal possibility thus appeared to be that respiration exerted selective effects on the metabolism of the diseased and healthy plants.

The changes due to prolonged respiration in the dark were therefore determined. For this purpose, twenty-four leaves were used in each series. Half of each leaf was removed for analysis to represent the original condition, the remaining half-leaves were left for 68 hours in the dark with their bases in water. The healthy and diseased leaves were similar in size and from corresponding levels of the stems. They were young leaves, not quite fully grown. Only a limited number of old healthy leaves was available. Six of these leaves were employed for comparison. The results are given in Table VII.

These results confirm the previous ones in showing a higher total N in the diseased leaves. It should be noted that, in these young leaves, the hydrolysable carbohydrate content is not greatly different, probably because photosynthesis had not been very active.

In regard to the changes in respiration, the carbohydrate loss on the dry weight basis is the same in both healthy and diseased leaves, but it is very noticeable that this loss falls chiefly on the insoluble carbohydrates in the healthy leaves, and mainly on the disaccharides in diseased leaves.

In this latter feature, the diseased leaves resemble the old healthy leaves rather than the young ones.

Table VII.

Type of leaf ...	Young healthy		Young diseased		Old healthy	
Treatment of half leaves	Original	68 hr. in dark	Original	68 hr. in dark	Original	68 hr. in dark
Insoluble N	4·456	4·052	5·009	4·775	2·489	2·489
Amino N	0·153	0·277	0·122	0·053	0·129	0·031
Rest N	0·597	0·851	0·680	0·786	0·546	0·547
Titratable acidity*	0·68	0·975	0·72	0·63	0·68	0·57
Insoluble carbohydrate	4·65	3·61	4·94	4·78	9·48	7·20
Disaccharide	4·47	4·21	3·77	2·49	7·78	5·42
Hexose	3·91	3·36	4·07	3·67	9·52	11·78
Total carbohydrate	13·03	11·18	12·78	10·94	26·78	24·40

Data as percentages of dry weight.

* As cc. of N/100 acid per 100 gm. dry weight.

The nitrogen fractions may most conveniently be considered from the following summary in which they are expressed as percentages of the total N.

Table VIII.

Type of leaf ...	Young healthy		Young diseased		Old healthy	
Treatment of half leaves	Original	68 hr. in dark	Original	68 hr. in dark	Original	68 hr. in dark
Insoluble N	84·0	78·2	86·3	85·1	78·7	81·15
Amino N	2·9	5·35	2·0	0·95	4·07	1·00
Rest N	13·1	16·45	11·7	13·95	17·23	17·85

The striking feature here is that, while the young healthy leaves show considerable breakdown of protein, followed by accumulation of amino N and subsequent increase in Rest N, the corresponding diseased leaves show much less protein breakdown, a marked *decrease* in amino N and a smaller increase of Rest N. In these features also they resemble the older healthy leaves.

It is to be noted also that there is a considerable increase in titratable acidity in the young healthy leaves, but a reduction of this acidity in the diseased leaves. In this also they resemble the older healthy leaves. On Ruhland and Wetzel's view that the common plant acids are largely produced from amino-acid oxidation, we could correlate the higher production of acidity in young healthy leaves with the larger proportions of protein broken down. In diseased and older leaves, however, the rate of loss of acids is evidently greater than the rate at which they are produced. Whether this interpretation is correct or not, the striking feature of all the results is the resemblance of diseased leaves to old leaves.

We may attempt an interpretation of these data on the assumption that protein hydrolysis yields amino acids which may then be oxidised, the ammonia formed during this oxidation being converted into Rest N (*i.e.* other soluble forms of nitrogen):

$$\text{Protein} + H_2O \rightleftharpoons \text{amino acids} + O \longrightarrow \text{Rest N.}$$

The greater increase in Rest N in healthy leaves may on this assumption be taken as evidence of more oxidation of amino acids and, from these results, the oxidation of amino acids would be in diseased leaves only about two-thirds what it is in healthy leaves, while the loss of protein is even less. In the older leaves both protein and amino acid losses are negligible.

Now, in the older leaves, the concentration of sugars is very much higher than it is in the younger ones, and it is a matter of experience that the sugars act as amino-acid sparers, they are oxidised more readily and also favour the synthesis of protein from amino acids. If we compare the ratio of amino acids to total sugars in these leaves, taking the average values over the period of the experiment, we get (as percentages of dry weight):

$$\text{Young healthy leaves } \frac{0{\cdot}215}{8{\cdot}0} = 0{\cdot}027.$$

$$\text{Young diseased leaves } \frac{0{\cdot}088}{7{\cdot}0} = 0{\cdot}012.$$

$$\text{Older healthy leaves } \frac{0{\cdot}065}{17{\cdot}2} = 0{\cdot}004.$$

That is to say that the ratio of amino acids to sugar is highest in the young healthy leaves and lowest in the older leaves, precisely the order in which the different leaves show increase in Rest N. Apparently then, the amount of amino acid oxidised depends on the ratio of amino acid to sugar. In the young leaves, it is clear that this ratio depends chiefly upon the amount of amino N present, and the lower values in the diseased leaves are due to the decrease in amino N. The ultimate factor controlling the slower rate of production of Rest N in diseased leaves is thus the slower rate at which the protein is hydrolysed. Evidently in the diseased leaves the amino acids tend to be used up in oxidation more rapidly than they are produced by hydrolysis. Protein breakdown as a whole is evidently slower in diseased leaves than in healthy ones.

DISCUSSION.

Considering now the general conclusions to be drawn from the data, it is noteworthy that in diseased leaves the insoluble fractions (that is, both proteins and hydrolysable polysaccharides, like starch) were less readily broken down. The conclusion to be drawn from this fact appears to be either that the enzymes responsible were present in small amount or else that they were less active. This conclusion is the more striking as the diseased leaves contain more protein, and a higher protein content is usually taken to imply a higher proportion of protoplasm.

The diseased leaves, as a result of the decreased hydrolysis, tend to become relatively deficient in both amino acids and disaccharides. Modern views tend to regard the latter as of especial importance in respiration, since they are thought to yield especially active sugars at the moment of hydrolysis. It would thus appear that there is an intimate metabolic similarity between the amino acids and disaccharides, since both readily yield substrates for respiration. On the basis of these results, it might be anticipated that the diseased leaves would show a rapid falling off and, ultimately, a lower respiration rate.

The young leaves used in the respiration experiment (Table VII) were chosen as representing leaves which were approaching the end of their growth period, and which were only at the beginning of their photosynthetic life. Since the differences due to the virus were fully developed, it follows that these differences are due to the effect of the virus on the growth metabolism. It is therefore appropriate to consider the growth metabolism of diseased leaves in reference to the conclusions already drawn. We have to account particularly for the slower rate of growth and the higher protein content of the infected plants. Since the rate of growth will be conditioned by the rate of supply of organised food materials from the mature leaves, the slower rate of hydrolysis of these substances in the diseased leaves would also indicate the possibility of a slower rate of growth. Further, since the same enzymes are presumably concerned in synthesis as are involved in hydrolysis, a lessened activity of these enzymes should also lead to slower growth. The possibility of a lower respiration rate in the diseased plants should also be considered in this connection. It is customary to regard the rate of respiration of a plant tissue as being proportional to the mass of protoplasm it contains, and to assume that the protein content (in an active tissue) is proportional to the amount of protoplasm. On this basis the diseased leaves have a relatively greater amount of protoplasm by from 15 to 30 per cent. To give the *same* respiration rate per unit of protoplasm, the diseased leaves would have to show a rate of respiration of from 15 to 30 per cent. greater per unit of dry weight. Now the results in Table VII show that the loss of carbohydrate per unit of dry weight was the same in both healthy and diseased leaves, while the increase in Rest N, which represents the amount of amino acid respired, was less in the diseased leaves. The indications are, therefore, that the rate of respiration per unit of protoplasm is less in diseased leaves. We would expressly point out, however, that the possible error of the carbohydrate estimations is sufficient to make this method of considering the respiration problem unsafe, and we quote the results merely to indicate that the possibility of a difference in respiration rate must be considered.

The data obtained were not designed to determine the effect of the virus upon photosynthetic activity. On comparing the four series of leaves, however, it may be noted that the young leaves (Table VII) represent the beginnings of photosynthesis while, in Table IV, the August leaves represent a period somewhat unfavourable for photosynthesis, the June and September series were for periods of high photosynthesis.

The total amounts of carbohydrate in these leaves per gm. of protein (Protein N × 6) were as follows:

	Young leaves		August		June		September	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Carbohydrate per gm. of protein	2·91	2·56	8·1	6·9	12·0	9·25	21·8	18·0
ratio	1·14 : 1		1·17 : 1		1·29 : 1		1·36 : 1	

It appears from the ratios of carbohydrates in diseased and healthy leaves that increase in the amount of photosynthesis increases the differences between the healthy and diseased leaves. This could be explained on the assumption that carbon assimilation is more active in the healthy leaves as found by Brewer, Kendrick and Gardner (2) in diseased leaves of tomato. Since the differences are already evident in the youngest leaves, it would seem on this assumption that carbon assimilation may start later or less rapidly in the diseased leaves. Barton-Wright and McBain (1) found that this was the case in potato infected with leaf-roll.

The relative accumulation of carbohydrates in relation to protein is, however, not only dependent on the relative rates of synthesis of these substances but also on the relative rates at which they are removed by respiration and translocation. The latter factor cannot of course be evaluated from these results, but the loss of protein by hydrolysis, and the rate of oxidation of amino acids, are both greater in healthy leaves for the same amount of carbohydrate loss (Table VII). These will lead to lower protein content in the healthy leaves, but as protein breakdown ceases when carbohydrates are abundant, they will not explain satisfactorily the increase in the differences when carbon assimilation is more active. It thus appears that the virus causes a depression of the rate of carbon assimilation.

While we may thus conclude that virus infection probably reduces the relative rate of carbon assimilation, there is no evidence to indicate whether this effect operates on the photochemical reaction or on the rate of conversion of the primary photochemical product into higher carbohydrate. As it has already been demonstrated, the rate of enzyme action is retarded, it is more probable that the second or protoplasmic reaction is affected. There is evidence that diseased leaves show disorganisation of their chloroplasts (Sorokin (12)) and that they also possess the photosynthetic pigments in different and possibly less proportions (Elmer (6)).

It seems, therefore, from the present results, that the primary effect of the disease is to retard the rate of action of hydrolytic enzymes. This would presumably account for the retardation of growth, and possibly it may also lead to slower photosynthesis. It evidently particularly affects protein hydrolysis, so that protein loss is much slower in the diseased leaves. Presumably protein synthesis might also be slower. Since the percentage of protein in a leaf decreases with age, a slower growth-rate may mean that, when samples of diseased leaves are collected, they may have a higher protein content than similar healthy leaves, since they may not have progressed so far along the normal metabolic path. In support of this idea of slower development is the observation (Grainger and Heafford (7)) that the rate of vacuolation is somewhat retarded in diseased leaves as compared with healthy ones. Against this view is the fact that diseased leaves in the dark behave more like old leaves than young healthy leaves. On the other hand, the supply of food to the growing tissues, if it resembles the soluble constituents in the leaves, will differ in the diseased plants from that in the healthy tissues. From the analyses given, it would contain a greater proportion of soluble nitrogen in diseased leaves. Under the influence of this different proportion of nitrogen, the growing tissues would tend to contain more protein and less carbohydrate.

The results of this examination of the metabolic effects of this mosaic disease are in marked contrast to those obtained by Sreenivasaya and Sastri (13) for the spike disease of sandal. This disease, which also induces a higher nitrogen content in the affected plants, is accompanied by a marked tendency towards greater protein hydrolysis and by much greater diastatic activity.

SUMMARY.

1. Analyses of healthy leaves and of leaves infected with mosaic disease (*N. tabacum*) show that the diseased leaves contain higher proportions of nitrogen and lower proportions of carbohydrate, these differences being apparently increased during photosynthesis.

2. In leaves kept in the dark for 68 hours, carbohydrate losses are similar but fall chiefly on insoluble carbohydrate in healthy leaves and on disaccharides in the diseased leaves.

3. In diseased leaves, protein breakdown is retarded and insoluble substances are less readily hydrolysed.

4. The diseased leaves resemble older leaves in their metabolism.

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THE MOVEMENT OF TOBACCO MOSAIC VIRUS WITHIN THE PLANT

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(With Plate IV and 4 Text-figures.)

Within the last few years several papers have appeared dealing with different phases of the movement of viruses through plants. Most of the work has dealt with viruses of the tobacco mosaic group (including tobacco mosaic, yellow tobacco mosaic, aucuba mosaic of tomatoes, and the mixed virus streak of tomatoes, one constituent of which is tobacco mosaic), but some of the conclusions arrived at have been decidedly conflicting. There have been two main views advanced with regard to the method of movement of tobacco mosaic virus: (1) that of a progressive advance from the point of inoculation through the tissues of the plant at a more or less uniform rate, and (2) that of a very slow cell to cell movement via the plasmodesmen combined with a rapid distribution through the plant via the phloem.

The former view was given its most definite expression in the work of Böning (1928), and has recently been supported by Caldwell (1930, 1931). The following quotations from Caldwell's papers give the main substance of this view of virus movement: "These results, which confirm the data of Böning, seem to indicate that there is no rapid movement of the virus agent in any one direction in the plant, but rather that the agent moves slowly up and down the stem from the point of insertion of the inoculated leaf" (1931, p. 293). "The rates of movement of the virus agent in the tomato are practically the same upward or downward. The slightly greater rate of upward movement appears to be associated with the greater metabolic activity which occurs in the upper portion of the plant" (1931, p. 297). "There is strong presumptive evidence that the movement of the agent of aucuba disease of tomato can take place and does take place readily through any living tissue, and that the phloem is, in this case, not the main channel of movement in the normal plant" (1931, p. 296). "It is concluded that movement takes place in the living ground tissue of the plant" (1930, p. 443)[1].

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[1] In a paper which appeared after this manuscript was submitted for publication Grainger (*Ann. Appl. Biol.* xx, 236) concluded that tobacco mosaic virus moves at a logarithmic rate, beginning slowly and later accelerating and also that spread seems to be independent of mechanical carriage by the transpiration or translocation streams.

On the other hand, the view of a rapid distribution of the virus through the plant via the phloem was expressed as early as 1898 by Beijerinck, and has recently gained much support from the experimental work of Holmes. Holmes (1930) found that there was an initial period of multiplication of the virus in local sites on the inoculated leaf, and then a comparatively sudden appearance of virus in all parts of the stem and root, and in the leaves at the top of the plant, the virus spreading later to lower leaves. Holmes pointed out that his technique using *Nicotiana glutinosa* might not be suitable for detecting very small traces of virus. But he presented a table showing measurements of virus in detached tomato stems which provided strong evidence that, if the virus had been present in one portion of the stem 2 or 3 days earlier than in another, his method would have detected it.

Holmes's work (1931, 1932) on the movement of mosaic within the inoculated leaf by means of the iodine test also showed an initial period when the virus was localised in primary lesions, and then a sudden appearance of virus all along the main vascular channel from the leaf. It was also shown that the first appearance of the virus in the systemically infected leaves was along the network of finer veins. Holmes made the suggestion that the restricted movement of the virus across the lamina of the inoculated leaf in comparison with its rapid movement down the mid-rib and through the stem might be explained on the basis of movement with food substances to dependent parts of the plant, and he pointed out that "if movement of virus with a particular food material could be proved the virus would serve as a useful indicator of the distribution of the substance." A number of his experiments on defoliation and the covering of leaves, and on inoculation of very young leaves, gave general support to these ideas.

It seems very likely that the cause of the discrepancy which exists between the experiments of Böning and Caldwell and those of Holmes is due to the fact that the former workers did not plan their experiments on a sufficiently large scale to be able to follow the movement of the virus during the first few days following inoculation. In the experiments quoted by Caldwell (1931, p. 292), for example, the position in which the virus was found by cutting up plants on the third day after inoculation may possibly have been assumed by movement from the inoculated leaf within a period only a few hours before the plants were cut up. Similarly, the majority of Böning's experiments are not inconsistent with Holmes's view of virus movement, but they are on too limited a scale to provide either confirmation or disproof. It seemed advisable, therefore, to under-take a more extended series of experiments with cuttings in order to see whether the results would fall into line with those determined by Holmes, using *N. glutinosa* as a test plant for the presence of the virus.

No fuller treatment of the literature on the movement of tobacco mosaic virus is attempted, since the papers of Böning (1928), Caldwell (1930) and Henderson Smith (1930) contain summaries up to recent dates.

EXPERIMENTAL.

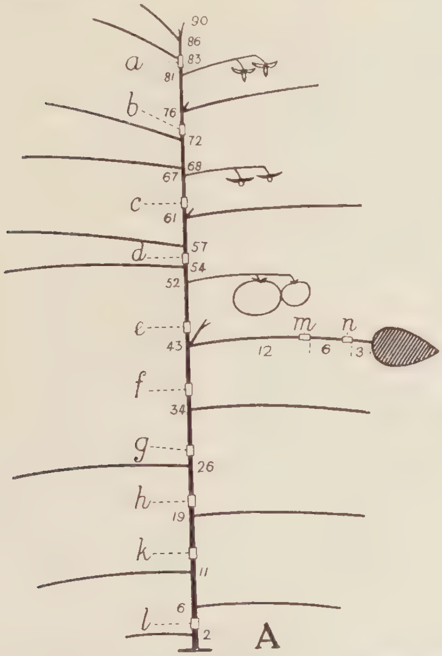
Comparison of methods.

A test was first made of the reliability of the various methods which have been used for determining the presence of the mosaic virus at different points within the plant. Three main methods have been used: (1) cutting up the stems (usually of tomato plants) at various times following inoculation and rooting the portions as cuttings; the cuttings are then grown until the presence or absence of mosaic can be determined from the symptoms on the new leaves; (2) cutting up the inoculated plants into sections, grinding these up and using the juice to inoculate seedlings of tobacco or tomato, the presence or absence of virus being judged according to the systemic infections which result; and (3) cutting into sections as for (2), but inoculating on to *N. glutinosa* leaves, and judging the presence (and roughly also the concentration) of virus from the number of local lesions produced.

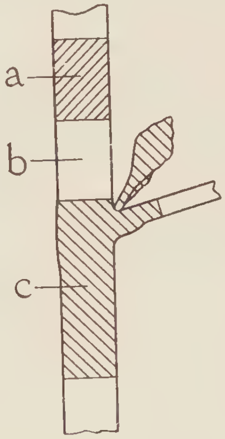
There seems little doubt that method (1) is entirely reliable, and that however small the amount of virus which gains entrance to a portion of a plant taken for a cutting, it will multiply and eventually be determinable from symptoms at the growing point. It is by no means certain, however, that a very small amount of virus in a portion of stem could be detected by the grinding up and inoculating methods (2) and (3). This was pointed out by Holmes (1930), but was apparently not considered by Böning (1928) in some of his experiments.

The matter was accordingly tested by using all three methods on the same series of plants. Ten tomato plants (*A*–*L*), each 100–120 cm. high and with about 20 leaves up the stem, were inoculated on the end leaflet of a leaf about half-way up the plant. Two plants were then cut up each day from the second to the sixth day according to the plan in Text-fig. 1 A. Before cutting up, a sketch of each plant was made and the heights of all the leaves recorded. A fresh sterile scalpel was used for cutting each sample, and each sample was handled only with a small square of clean newspaper in the hand, so that at no time was any portion of the plant touched with the fingers. The pot containing the base of the stem, with one or two buds left, was always kept and grown on to determine whether the virus had passed beyond the lowest cut, probably into the roots.

Cuttings were made from five portions of the stem above the insertion of the inoculated leaf and from five portions below. Each cutting necessarily contained an axillary bud, with about 2 cm. of stem below (Text-fig. 1 B, *c*); the sketch-plan gave the distances between each. Each cutting was immediately planted in a (5 : 1) sand-soil mixture in a 4 in. pot, and comparatively few cuttings failed to root and grow. In cases when the cutting did fail to grow, a grinding-up test with inoculation to *N. glutinosa* revealed the presence or absence of virus.



A



B

Text-fig. 1 A. Working plan for plant 32 in Table III, showing height in cm. of every leaf and fruit truss, and the positions (*a–l*) from which portions of the stem were taken for tests for the presence of virus. Petiole samples from *m* and *n*, with distances from the inoculated leaflet. Similar plans were made for every plant cut up.

Text-fig. 1 B. Plan for one node of the stem in the case of the plants in Tables I and II, showing (*a*) portion taken for the "test-tube" method, (*b*) portion taken for an immediate expressed sap test on tobacco or *N. glutinosa*, and (*c*) portion rooted as a cutting.

Expressed sap tests for the presence of the virus were made by taking small pieces of stem about 1 cm. long from immediately above the portions used for cuttings (Text-fig. 1 B, *b*). These were ground up in a mortar with five drops of water, and inoculated with a small glass spatula on to two leaves of small tobacco seedlings and on to single leaves of *N. glutinosa* plants.

During the course of the experiment a fourth method was thought of and applied to plants *G* to *L* in addition to the other methods. This consisted in taking a further series of 1 cm. lengths of stem, adjacent to the other samples (Text-fig. 1 B, *a*), and placing them in sterile test-tubes with a little moistened cotton-wool at the bottom. The samples were kept for a week in a well-lighted room, when they were ground up and inoculated on to single leaves of *N. glutinosa* plants. It was considered that a very small amount of virus which had just arrived in a portion of stem might be missed by methods (2) and (3), but that it would have an opportunity to multiply during the week in a test-tube and would then be easily detectable by the *N. glutinosa* test. The principle is much the same as that used in the method of cuttings, but it has the advantages (1) that the necessity of taking an axillary bud and a moderate length of stem (2–3 cm.), as necessary for the cuttings, is dispensed with; (2) that there is a great saving in time, since the results are available in about 10 days, whereas with cuttings it is often necessary to grow them on for 6 weeks or more before all the results can be recorded; and (3) there is thus a considerable saving in greenhouse space. This fourth method is in essence a "tissue culture," and was previously used by Holmes (1930) in tests on the rate of multiplication of virus.

The results of the whole experiment are presented in Table I. It is evident that the method of cutting up the stem into small portions, which are immediately ground up and inoculated on to tobacco plants or on to *N. glutinosa* plants, does not give a reliable indication of whether virus had reached the particular parts under test or not. In plant *L*, for example, no sign of virus was detected by the expressed sap tests, either on *N. glutinosa* or on tobacco, and yet the cutting tests later showed that the virus was present throughout the whole of the stem. Apparently the number of virus particles present could be so small that either the chance of their introduction to a suitable inoculation wound on the test plant was almost nil, or they were rendered ineffective by adsorption effects when ground up with the whole bulk of tissue. In plant *K* a few positive tests resulted (one on *N. glutinosa*, four on tobacco), but here again the virus was actually present throughout the whole of the stem. It had probably been present for 1, or possibly for 2 days, and had multiplied sufficiently in certain places to be detected by expressed sap tests. (The number of local lesions on the *N. glutinosa* leaves in these cases was very low.) It is seen, on the other hand, that the results from keeping the stem samples in test-tubes for a week before crushing them to test for the presence of virus on *N. glutinosa* leaves gave results identical with those given by the cuttings.

Table I.

A comparison of four methods for determining the presence of the first small traces of tobacco mosaic virus which penetrate the stem of a tomato plant following inoculation on a leaf about half-way up the stem.

Plant no.	Days after inoculation	Stem sample (see Text-fig. 1)									
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*
Expressed sap tests on *N. glutinosa* (Method 3).											
C	3	–	–	–	–	–	–	–	–	–	–
D	3	–	–	–	–	–	–	–	–	–	–
E	4	–	–	–	–	–	–	–	–	–	–
F	4	–	–	–	–	–	–	–	–	–	–
G	5	–	–	–	–	–	–	–	–	–	–
H	5	–	–	–	–	–	–	–	–	–	–
K	6	–	–	–	–	–	–	–	–	–	+
L	6	–	–	–	–	–	–	–	–	–	–
Expressed sap tests on *N. tabacum* (Method 2).											
C	3	–	–	–	–	–	–	–	–	–	–
D	3	–	–	–	–	–	–	–	–	–	–
E	4	–	–	–	–	–	–	–	–	–	–
F	4	–	–	–	–	–	–	–	–	–	–
G	5	–	–	–	–	–	–	–	–	–	–
H	5	–	–	–	–	–	–	–	–	–	–
K	6	–	–	–	–	–	+	–	+	+	+
L	6	–	–	–	–	–	–	–	–	–	–
Tests by rooting cuttings (Method 1).											
C	3	–	–	–	–	–	–	–	–	–	–
D	3	–	–	–	–	–	–	–	–	–	–
E	4	–	–	–	–	–	–	–	–	–	–
F	4	–	–	–	–	–	+	+	+	+	+
G	5	–	–	–	–	–	+	+	+	+	+
H	5	–	–	–	–	–	+	+	+	+	+
K	6	+	+	+	+	+	+	+	+	+	+
L	6	+	+	+	+	+	+	+	+	+	+
Test-tube samples 1 week old on *N. glutinosa* (Method 4).											
G	5	–	–	–	–	–	+	+	+	+	+
H	5	–	–	–	–	–	+	+	+	+	+
K	6	+	+	+	+	+	+	+	+	+	+
L	6	+	+	+	+	+	+	+	+	+	+

Other points of interest in Table I are (1) that there was no movement of virus into the stem before the fourth day, and (2) that when virus first appeared in the stem it went downwards towards the roots very quickly, and then, about a day later, went equally quickly up to the top of the stem. The number of plants was certainly small, but in none was the virus intercepted when it had only travelled a part of the way up or down the stem.

Returning to the question of methods, because of the success of the test-tube method and the saving in time and glasshouse space made possible by its use, a confirmatory test was carried out, the results of which are presented in Table II.

Table II.

A further examination of the "test-tube method" for detecting the first traces of virus entering the tomato stem. Plants cut up 5 days after inoculation.

Plant no.	Stem sample (see Text-fig. 1)									
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*
Stem samples tested on *N. glutinosa* on day of cutting up.										
M	−	−	−	−	−	−	−	−	−	−
N	−	−	−	−	−	−	−	−	−	−
P	−	−	−	−	−	−	−	−	−	−
Q	−	−	−	−	−	−	−	−	−	−
R	−	−	−	−	−	−	−	−	−	−
Stem samples preserved in test-tubes 1 week and then tested on *N. glutinosa*.										
M	+	+	+	+	+	+	+	+	+	+
N	+	+	+	+	+	+	+	+	+	+
P	+	+	+	+	+	+	+	+	+	+
Q	+	+	+	+	+	+	+	−	+	+
R	−	+	+	+	−	+	+	+	+	−
Cuttings rooted from the nodes.										
M	+	+	+	+	+	+	+	+	+	+
N	+	+	+	+	+	+	+	+	+	+
P	+	+	+	+	+	+	+	+	+	+
Q	+	+	+	+	+	+	+	+	+	+
R	+	+	+	+	+	+	+	+	+	+

This test once again showed the inadequacy of results obtained from crushing samples of stem at the time of the cutting up of plants as a method of determining the presence or absence of the first traces of virus in the portions taken. In none of the samples taken immediately on crushing could any virus be detected, yet it must certainly have been present since in the adjacent stem samples kept in test-tubes for 1 week before crushing abundant virus was found in nearly every case.

There were, however, four cases of negative results from the test-tube samples, whereas all the cuttings gave positive results. It is difficult to see why a negative result should have been obtained if there was any virus present in the sample taken, for the portions of stems in the test-tubes were under just as favourable conditions as the cuttings and the great majority of them showed multiplication of virus. It is just possible that in these cases the virus for some reason did not move out from the phloem to the mesophyll tissue. The only other explanation is that these samples were actually free from virus particles when taken, and that there was no virus there to multiply. There is further evidence to support this last conclusion, and the matter is discussed again later. It may be noted here, however, that the samples which gave negative results were from internodes, whereas the cuttings necessarily included nodes, and it seems possible that the vascular twists at the nodes would have a greater tendency towards the temporary arrest of widely spaced virus particles passing down vascular channels such as the phloem.

MOVEMENT OF VIRUS FROM THE INOCULATED LEAF INTO THE STEM.

Experiment 1.

After the experience gained from the preliminary experiments it was decided to use the test-tube method for the first larger experiment. Forty tall tomato plants (Dwarf Champion variety), growing in 8 in. pots and 70–90 cm. in height, were inoculated with mosaic on the end leaflet of a leaf about half-way up the plant (Text-fig. 1 A). The inoculations were done at 9–10 p.m. at night, and then commencing at 9–10 a.m. on the next day, but one group of four plants was cut up into sections at intervals of 12 hours. Again ten samples were taken at various distances up the stem, five above the inoculated leaf and five below. The samples were 1 cm. portions of stem cut with sterile scalpels and placed in sterile test-tubes on moist cotton-wool for 1 week. The same methods of handling were adopted as previously described, with the addition that in this case two samples (*m* and *n*) were taken at measured distances down the petiole below each inoculated leaflet. All samples were ground up 7–10 days later and inoculated on to single *N. glutinosa* leaves. If virus was present in a stem sample, some twenty to eighty local lesions were found on the inoculated *N. glutinosa* leaf, otherwise there were none. The results of the experiment are given in Table III.

The following are the main points of interest in Table III:

(1) With one exception (at $3\frac{1}{2}$ days) no virus moved out of the inoculated leaf into petiole or stem until the fourth day.

(2) In six plants (23, 25, 27, 28, 31, 35) the virus travelled down to the roots immediately it left the inoculated leaf.

(3) In three other plants (32, 33, 36), as well as moving down to the roots it also moved up one or two internodes. In plant 32, where it moved up one internode, a developing fruit truss was situated one internode above the insertion of the inoculated leaf (the diagram in Fig. 1 A

refers to this plant), and in plants 33 and 36, in which it moved up two internodes, developing fruit trusses were situated two internodes above the insertion of the inoculated leaf. On the other hand, certain of the six plants in which the virus only moved downwards had also developing fruit trusses one internode (plant 35) or two internodes (plants 25, 27) above the insertion of the inoculated leaf. It would therefore seem that the normal path of the virus on leaving the leaf is down to the roots, but that the presence of a developing fruit truss a few nodes above the insertion of the inoculated leaf may sometimes exert a pull causing part of the virus to move upwards as far as this point in addition to the normal downward movement.

Table III.

Results of cutting-up tests on tomato plants inoculated with tobacco mosaic on a leaf about half-way up the stem, showing determinations for the presence of the first traces of virus in the stem by the test-tube method. Dwarf Champion plants, 70–90 cm. high, bearing several fruit trusses.

Plant no.	Days after inoculation	Stem sample (see Text-fig. 1)										Base of plant	Petiole	
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*		*m*	*n*
1–4	1½	–	–	–	–	–	–	–	–	–	–	–	–	–
5–8	2	–	–	–	–	–	–	–	–	–	–	–	–	–
9–12	2½	–	–	–	–	–	–	–	–	–	–	–	–	–
13–16	3	–	–	–	–	–	–	–	–	–	–	–	–	–
17–19	3½	–	–	–	–	–	–	–	–	–	–	–	–	–
20	3½	–	–	–	–	+	–	–	–	–	–	–		–
21	4	–	–	–	–	–	–	–	–	–	–	–	–	–
22	4	–	–	–	–	–	–	–	–	–	–	–	+	+
23	4	–	–	–	–	–	+	+	+	+	–	+	+	–
24	4	–	–	–	–	–	–	–	–	–	–	–	–	–
25	4½	–	–	–	–	–	+	–	+	–	+	+	–	–
26	4½	–	–	–	–	–	–	–	–	–	–	–	–	–
27	4½	–	–	–	–	–	+	+	+	–	–	+	–	–
28	4½	–	–	–	–	–	+	+	+	+	+	+	+	+
29	5	–	–	–	–	–	–	–	–	–	–	–	–	–
30	5	+	+	+	+	+	+	+	+	+	+	+	+	+
31	5	–	–	–	–	–	+	+	+	+	+	+	+	+
32	5	–	–	–	–	+	+	+	+	+	+	+	+	+
33	5½	–	–	–	+	+	+	+	+	+	+	+	+	+
34	5½	+	+	+	+	+	+	+	+	+	–	+	+	+
35	5½	–	–	–	–	–	+	+	+	+	+	+	+	+
36	5½	–	–	–	+	+	+	+	+	+	+	+	+	+
37	6½	–	+	+	+	+	+	+	+	+	+	+	+	+
38	7½	+	+	+	+	+	+	+	+	+	+	+	+	+
39	8½	+	+	+	+	+	+	+	+	+	+	+	+	+
40	9½	+	+	+	+	+	+	+	+	+	+	+	+	+

(4) In one case (plant 20) the virus moved upwards one internode, where there was a developing fruit truss, before it moved downwards.

(5) In plant 37, where the virus was not found in the highest sample, the top of the plant had been scorched one hot day, and the main growth

was being resumed at a side-shoot just below where the highest sample was taken.

(6) In plants 23, 25, 27 and 34 negative tests were obtained on stem-samples situated *between* positive tests, suggesting that the virus must have passed *through* these portions of stems without affecting them. Similar results were obtained in a number of cases with cuttings, and there seems little doubt that the suggested explanation is correct. The subject is further discussed below.

(7) The same applies to the petiole tests in plants 23, 25 and 27.

(8) In one case only (plant 22) was virus found in the petiole but not in the stem or roots. In all other cases when once the virus had left the inoculated leaflet it was found (occasionally at intermittent points only) right down to the roots, and about a day later up to the top of the stem as well. It must therefore have moved with considerable speed.

(9) No differences could be observed between the morning and night samplings, but the number of plants in which any such differences could have been observed was very few.

(10) It may fairly be concluded from the results in Table III, combined with similar results from several other experiments, that plants showing virus present throughout the whole stem (such as plants 30, 34, 37, 38, 39 and 40) have already passed through the initial stage when virus moved downwards only.

Experiment 2.

Because of the unexpected nature of the results of the preceding experiment, and especially because certain stem samples gave negative results when the virus must have passed through them, it was decided to repeat the experiment using the method of planting the stem sections as cuttings. It was considered that, if the two methods gave similar results, there would be little doubt as to the reality of the happening.

In this case nine plants were cut up on the third, fourth and fifth day and five plants only on the sixth day after inoculation. The plants were of the tall variety Sensation, 100–140 cm. high. The cuttings were necessarily nodal, with 2–3 cm. of stem, and each was grown in a separate 4 in. pot as before. The results of this experiment are given in Table IV.

In this case it was only in certain plants cut up on the fourth day after inoculation that the early stages of invasion of the stem were intercepted. (Possibly this was due to the fact that this was the most vigorously growing batch of plants used.) Once again the tendency for the virus first to pass downwards towards the roots was evident (plants 13, 14 and

15). In plants 15 and 16, in which the virus was also found to have moved upwards some distance, small developing fruit trusses were situated just above the highest positive record, but this may have been only a coincidence. In two cases (plants 14 and 15) the virus was intercepted before it had reached the lowest section of the stem or the roots.

Table IV.

Results of cutting-up tests on tomato plants inoculated with tobacco mosaic on a leaf about half-way up the stem, showing determinations for the presence of the first traces of virus in the stem by the method of rooted cuttings. Sensation tomato plants, 100–140 *cm. high, bearing several fruit trusses.*

Plant no.	Days after inoculation	Stem sample (see Text-fig. 1)										Base of plant	Petiole*	
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*		*m*	*n*
1–9	3	–	–	–	–	–	–	–	–	–	–	–	–	–
10	4	–	–	–	–	–	–	–	–	–	–	–	–	–
11	,,	–	–	–	–	–	–	–	–	–	–	–	–	+
12	,,	–	–	–	–	–	–	–	–	–	–	–	–	–
13	,,	–	–	–	–	–	+	–	–	–	+	Died	–	+
14	,,	–	–	–	–	–	+	+	+	+	–	–	–	–
15	,,	–	–	–	+	–	+	+	–	+	–	–	–	–
16	,,	–	+	+	+	+	+	–	–	–	–	+	+	+
17	,,	+	+	+	+	+	+	+	+	–	+	+	+	–
18	,,	+	+	+	+	+	+	+	+	+	+	+	–	–
19–27	5	+	+	+	+	+	+	+	+	+	+	+	+	+
28–32	6	+	+	+	+	+	+	+	+	+	+	+	+	+

* The tests on the two portions of the petioles were done by the test-tube method.

In plants 13, 15, 16 and 17 there was again definite evidence of the virus having passed through sections of stem without causing any infection. There was no doubt about these negative records, for in six such cases the cuttings were transplanted to richer soil after having rooted in the sand, and when they still appeared healthy after having grown to a height of about 9 in. a final test with *N. glutinosa* was made upon them. In the case of the negative result for cutting *k*, plant 17, there seems no other conclusion possible than that the virus had passed through this section of the stem without infecting it. In the case of plant 16, where the virus had passed through four consecutive samples without infecting them and yet was found in the base of the plant, it would be reasonable to suspect the accidental infection of the basal section. Every possible precaution, however, was taken to avoid accidental infections, and it is not believed that this is the explanation. The frequency with which the virus was found to have passed through one or two consecutive segments without infecting them, not only in this but in other experiments, would make it not unreasonable to extend the same view of virus movement to cover this case of plant 16. The distance between the bases of samples *f* and *l* in this plant was 50 cm., but each cutting was only 2½ cm. long, which means that only 10 of the 50 cm. of stem was actually tested for the presence of virus. It is possible that virus would have been found at intermediate points if the whole 50 cm. had been tested. Also it is again noticeable how frequently tests on the petioles of the inoculated leaves (by the test-tube method) gave negative results when the virus was already in the stem.

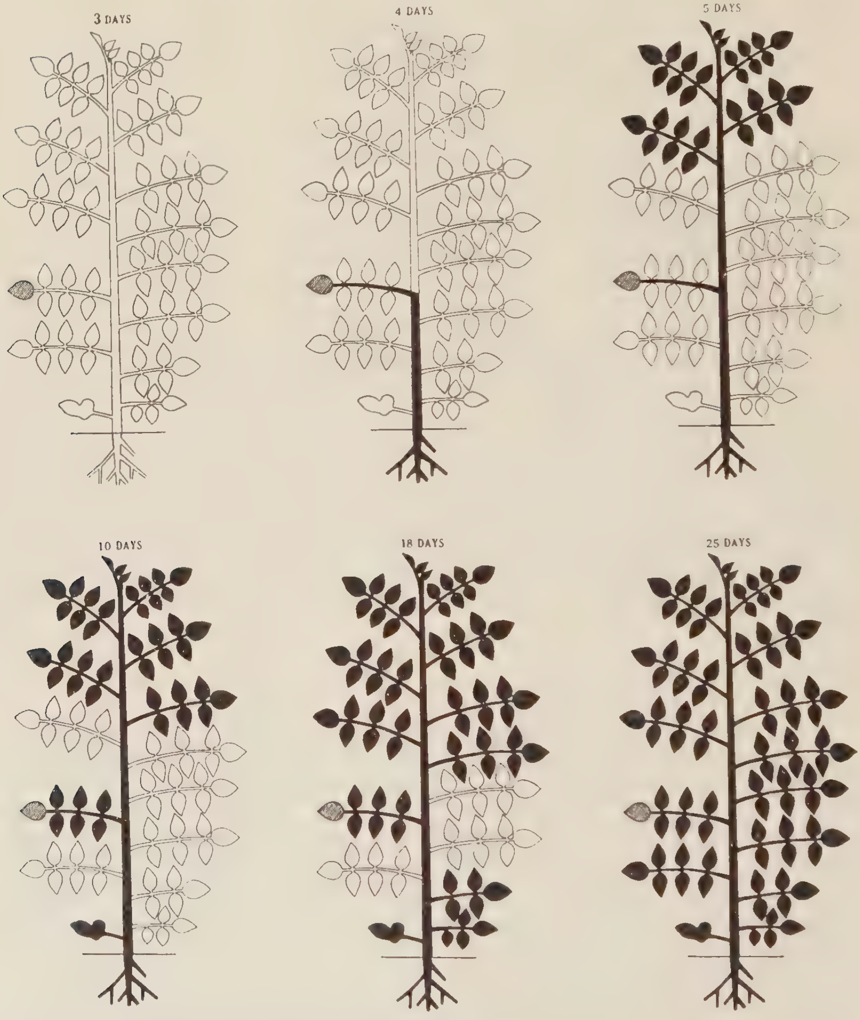
Several further experiments were done both by the cutting and by the test-tube method. These gave essentially the same results, except in one case. In this instance, which occurred in one of the test-tube series, the virus was found in four of the top five samples, but not in the lower five. This was the only case out of some dozens in which the virus was found to have moved first upwards in the stem instead of downwards, there being no fruit trusses on the plant. It is just possible that there was a mistake in the ticketing of the *N. glutinosa* plants used, but that is not thought likely. The plant was in a series of young Sensation tomatoes averaging 50 cm. high and not yet flowering; they were in 6 in. pots and commencing to suffer from lack of nitrogen. (There was no movement of the virus out of the inoculated leaves in this series until 5–7 days.) One other plant in the same series gave the usual initial downward movement from the inoculated leaf.

MOVEMENT INTO THE LEAVES.

Holmes (1930) has already presented tables showing that the virus, after leaving the inoculated leaf a few days following inoculation, appears suddenly in the stem, in the leaves at the top of the plant, and in the roots, almost simultaneously, but that it does not invade the central leaves on the stem until later. These tests were done by crushing samples and inoculating immediately on to *N. glutinosa*, and, as previously shown, this method suffers from the disadvantage of only detecting the virus a day or so after it is actually there.

That this disadvantage actually does apply to leaf, as well as to stem samples, was proved by taking opposite leaflets from several leaves towards the top of a plant shortly after systemic infection had occurred but was not yet visible, and testing one lot on *N. glutinosa* leaves immediately, while the other lot was kept in test-tubes for a week before testing. It was found that the lot kept in test-tubes showed the virus to be present on the average one leaf lower down the plant than could be detected by the test done immediately on sampling.

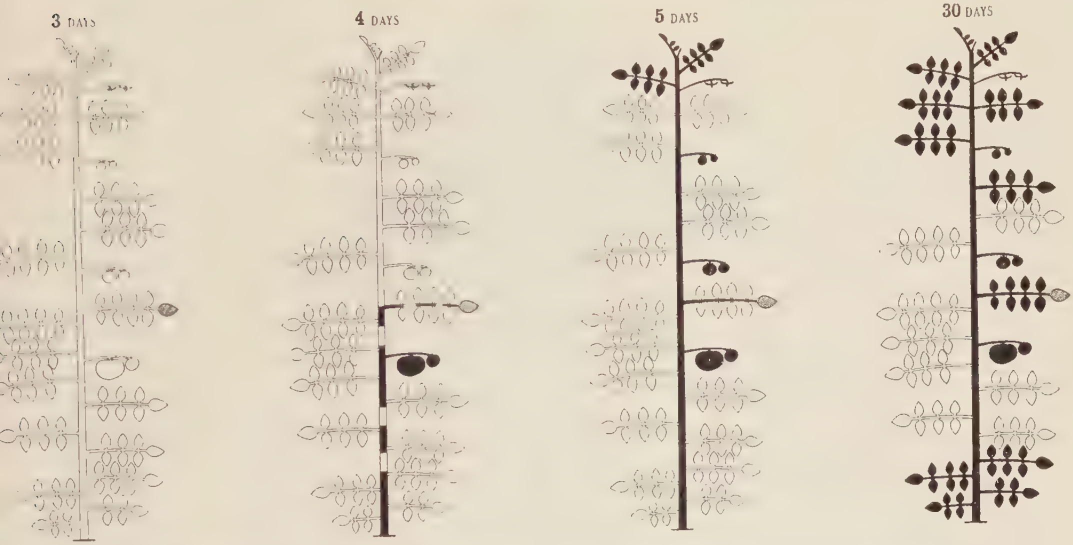
It was also proved by taking every leaflet from several leaves about the level where invasion was just occurring, that when the virus moves into a leaf it moves into all the leaflets almost simultaneously (within about a day). This made it legitimate, in tests for the distribution of virus throughout the leaves of a plant, to take a single leaflet from each



Text-fig. 2. Diagram to show the progress of the spread of mosaic (in black) through a medium young tomato plant. Based on tests of Dwarf Champion tomato plants about 15 in. high, growing in 6 in. pots in an unheated greenhouse. Inoculated leaflet shaded.

leaf, thus permitting determinations to be made again later on the same plants, and more than once, if necessary. The rate of invasion of the leaves in the middle of the stem could thus be followed on a single plant.

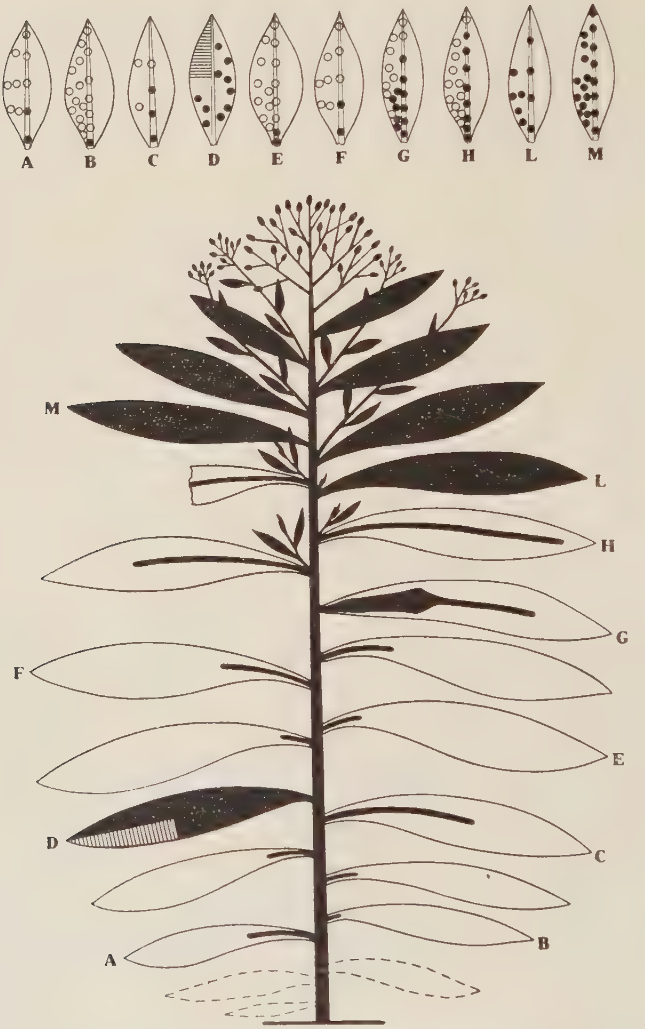
The results obtained from sampling every leaf of tomato plants of various ages confirmed entirely the work of Holmes. They are therefore not given in detail but are summarised graphically in Text-figs. 2 and 3.



Text-fig. 3. Diagram to show the progress of the spread of mosaic (in black) through an older fruiting tomato plant. The intermittent blackening at 4 days merely expresses the fact that the earliest virus particles to pass out from the inoculated leaflet are found at intermittent distances down the stem. The stem, of course, is not infected right through in these places; a mental picture at such a stage should imagine the infective particles (represented by the black areas) moving slowly down to the roots and gradually becoming more and more numerous. Similarly, although the fruit is entirely blackened, in reality only very few virus particles would have yet entered it. Inoculated leaflet shaded.

Young plants are completely invaded by the virus very soon after the virus becomes systemic; medium plants are often not fully invaded until some 3 weeks after inoculation (Text-fig. 2); and old plants may not be fully invaded 1 or 2 months or more after inoculation (Text-fig. 3).

Tests made on field plants, which were much larger, showed that there was still less distribution of virus throughout the plants than occurred in the oldest of the plants in pots. Text-fig. 4 shows the approximate distribution of virus in a tobacco plant *three months* after inoculation, and it is



Text-fig. 4. Diagram to show the approximate distribution of mosaic virus (in black) in a field tobacco plant 3 months after inoculation. 150 samples to test for the presence or absence of virus were taken with sterile cork borers at various points; the position of most of these is indicated by circles in the set of leaves above, the circles being blackened where virus was found to be present. The tobacco plant was 6 ft. high and most of the leaves 2 ft. in length. Inoculated quarter-leaf shaded.

seen that, beyond a slight creeping out into the basal parts of the midribs, there appears to have been no tendency whatever for the virus to move into the mature leaves. This plant was inoculated before flowering, and the capsules were nearly ripe at the time the tests were made. Tests made on other field plants, both tobacco and tomato, confirmed this result, and showed that when the plant inoculated was already a few feet in height, invasion of the stem and of the leaves at the growing point occurred within 12 days, but that the mature leaves (apart perhaps from some near the top) remained free from virus until the time of testing some 2 months later.

It seems possible that the difference between the spread of virus in field plants as compared with pot plants may be connected with the fact that the leaves of pot plants are rarely fully grown, so that organic food materials may continue entering them for some time. The leaves of the field tobacco plant in Text-fig. 4 were from 20–26 in. long, so that they were undoubtedly mature, and movement of assimilates in the phloem may have been entirely away from the leaves.

Nelson (1932) has recently drawn attention to the fact that systemic infection of plants by viruses may often not be so complete as was formerly supposed.

MOVEMENT INTO THE FRUIT.

As shown in Text-fig. 3, the virus was found to move into all the developing fruit trusses up the stem of a tomato plant immediately on invasion of the stem. This was in marked contrast to the long time taken for invasion of the adjacent leaves.

DISCUSSION.

The results of the foregoing experiments are definitely in favour of the phloem theory of distribution of the virus of tobacco mosaic through the plant.

Speed of movement. It is evident from Table III that the virus travels from the inoculated leaf down to the roots in considerably less than 12 hours. Using the methods employed in this work it would be almost impossible to determine the exact speed of transport, since, by the method of mechanical inoculation of mesophyll cells, the time at which the virus enters the phloem is never known precisely. If a method could be obtained for injecting the virus directly into the phloem, the speed of movement should be easily ascertained. The fact that *Myzus pseudosolani* will transmit tobacco mosaic from tomato (Hoggan, 1931) offers a promising starting-point for work of this type.

At all events it appears probable that the rate of movement of tobacco mosaic virus within the plant may well fall into line with the high rates of movement already determined for sugar-beet curly top and maize streak. Severin (1924), working with the curly top virus, determined a speed of 7 in. (17·8 cm.) in half an hour at a temperature of 39·7° C. Storey (1928) found in one experiment that the virus of maize streak travelled 40 cm. in 2 hours at a temperature of 30° C. It is generally agreed that the rapid rate of movement of these insect-inoculated viruses from the moment of inoculation is probably due to the virus being injected directly into the phloem. It is significant that the evidence we possess on the rate of movement of metabolites through the phloem gives figures of the same order. Calculations on the rate of movement through the phloem which would be required to account for the observed increase in dry weight of developing fruits and tubers (Dixon, 1923; Mason and Lewin, 1926; Crafts, 1931) have given figures ranging from 20 to 50 cm. or more per hour. Crafts, moreover, determined a rate of about 0·3 cm. per min. (18 cm. per hour) from phloem exudation measurements, using cut stems of Cucurbitaceae, and Mason and Maskell (1928) found that changes in sugar concentration in the leaf could be reproduced within a period of 2 hours in the bark at a minimum distance in one experiment of about 50 cm.

Transport in the early stages as separated particles. The distances which must occasionally separate some of the earliest particles of virus that travel down the phloem are of interest. Since in the present experiments only 2½ cm. samples were taken at 5–10 cm. intervals down the stem, it is not possible to state the maximum distance (beyond 2½ cm.) which separated particles of virus in any particular case. As an extreme case it seems quite conceivable, however, that under certain conditions a few virus particles might escape into the phloem and be carried down as far as the roots without infecting any of the tissues on the way, before the next particles entered the phloem.

Escape of virus from the phloem. At present we have no tests delicate enough to detect the presence of the virus while it is still in the phloem, and before it has escaped into the adjacent mesophyll cells apart from the method of taking cuttings or test-tube samples from the stem. Plate IV, fig. 1, shows a leaf inoculated with yellow tobacco mosaic just above a gap in a lateral vein. The leaf was killed and stained with iodine 6 days after inoculation, and systemic symptoms developed the following day on the plant from which it was removed. The virus must therefore already have passed from the primary lesion through numerous small veins round the gap and then down the mid-rib into the stem, but no sign of it could be detected by the iodine test in the inoculated leaf, beyond the primary lesion. Nor could it be detected in certain other similar cases by direct sampling of the tissue below the gap, with subsequent inoculation. The virus could only be detected when it had already passed out of the phloem, presumably through lateral sieve plates, and had multiplied for a little while in the adjacent mesophyll cells (Plate IV, figs. 2, 3).

The passing out of the veins from the phloem may frequently occur at fairly widely separated points. This may be seen in the small isolated spots of virus multiplication which are visible in iodine stained leaves: (1) in early stages of systemic invasion of young leaves (Holmes, 1931, Fig. 2G); (2) when the inoculation point on a leaf has been between the main lateral veins instead of near them (Holmes, 1931, Fig. 2D); (3) when the inoculation point has been over a lateral vein, but the virus has been forced by cuts to take a path through small veins (Holmes, 1932, Fig. 3D, and this paper, Plate IV, figs. 2, 3, 4); and (4) when *N. glauca* is inoculated with yellow tobacco mosaic. In this case, although abundant infection can be obtained on the inoculated leaves when sand is used in the inoculum (Samuel and Bald, 1933) and the virus multiplies considerably in the primary lesions, the virus appears to be unable to move out of the inoculated leaf except on rare occasions. In the few cases in which escape does occur the virus becomes visible on the upper leaves in small yellowish spots situated in intimate relation with some of the smaller veins (Plate IV, fig. 5). It can be shown that these spots are local sites of multiplication of the yellow tobacco mosaic, and that no virus is detectable in the intervening tissues of the leaf. This unusual case could be understood if an exceptional penetration of a few virus particles from the primary lesions into the phloem occurred, these, when carried up to higher leaves, passing out into mesophyll cells where it is known that they can multiply readily. A somewhat similar type of case is dealt with below when discussing the effect of temperature on viruses which produce necrosis.

Effect of temperature on virus movement. No extended discussion of the effect of temperature on the movement of virus can be undertaken here, but it is desired to refer to one case only, namely, that of tobacco mosaic on *N. glutinosa*. It is well known that at ordinary temperatures the primary lesions formed by tobacco mosaic on this host are necrotic, and that the virus does not occur in the intervening green parts of the leaf, nor become systemic in the plant. The virus apparently causes such a severe necrosis in the cells that they collapse and die before there is much multiplication of the virus, or before there is any possibility of its entering the phloem. Nevertheless, as recorded previously (Samuel, 1931), at a temperature of 35° C. the primary lesions are not necrotic, there is abundant multiplication of virus, and systemic infection takes place in the normal manner. This relation between low temperatures and increased necrosis, and *vice versa*, applies also to other cases among plant virus diseases.

Sometimes when *N. glutinosa* plants are inoculated with tobacco mosaic in the greenhouse there is no movement of virus from the inoculated leaves for some time (beyond a very slow spread of the necrosis down the petioles to the basal part of the stem), and then suddenly a small portion of the stem, or of an upper leaf, will become necrotic as if the virus had travelled upwards without affecting the intermediate tissue. Caldwell (1932) recorded the same thing for aucuba mosaic on *Datura stramonium*. A possible explanation of this is that a rise in temperature, such as might occur in a glasshouse on a sunny day, would allow of multiplication of virus without necrosis in some cells bordering a sieve-tube; a few virus particles might then enter the phloem and be carried upwards; on passing out into mesophyll cells a short period of multiplication might occur, and then necrosis supervene following a fall in temperature.

In cases such as the above, no virus can usually be detected in the stem between the upper and the lower necrotic patches, probably because no virus is there; the virus particles may have passed into and along the phloem only during a short period when the temperature made it possible.

The evidence which has been gathered above is very definitely in favour of the long distance transport of tobacco mosaic virus through the plant being accomplished via the phloem. This would bring the tobacco mosaic virus into line with many other viruses, especially insect-transmitted viruses, which are already fairly generally recognised as being distributed via the phloem (Quanjer, 1931, p. 581).

Since tobacco mosaic is in many ways a particularly easy virus to work with, it offers attractive possibilities as an aid in the study of phloem movements, and in this way may contribute a chapter to plant physiology which would be very welcome. The observation that the virus almost invariably moves down to the roots first, except when developing fruit are situated a little above the inoculation point, is of particular interest. If it is a true indication of the path of movement of metabolites from the leaf, it is a fact which could have been determined in no other way as yet.

The subsequent movement to the top of the plant would also suggest that the metabolites used at the growing point came, not directly from the leaves, but via the roots.

These results can only provide a stimulus for further work, both with tobacco mosaic and other viruses. More complete studies on the movement of the numerous potato viruses would be particularly valuable, but it is obvious that to be of use these must be planned on a much more extensive scale than has been done in the past, including adequate determination of the position of the virus in the early stages following inoculation. Kunkel (1930) recorded that the virus of peach yellows moves down the stem about ten times as fast as it moves upwards. Full details of these experiments have not yet appeared, but there would seem to be some other factor than ploem movements concerned in this case. Possibly particle size of the virus is important. It is these cases which apparently cannot be explained purely on the basis of phloem transport that are the most interesting.

At first sight it would seem that what have been called the different rates of travel of the two constituents of certain mixed viruses (such as tomato streak) might prove rather difficult to explain by the phloem theory of transport. But it has not yet been satisfactorily demonstrated that there actually *are* different rates of travel of the two constituents of these mixed viruses. It has only been demonstrated that there are different times of arrival at a point at some distance from the inoculation point. Differences in rate of multiplication of the two viruses in the primary lesion, or of size of particles, might determine very different times of entry of each into the sieve-tubes.

SUMMARY.

By means of extensive cutting-up tests on tomato plants inoculated with tobacco mosaic the following points have been determined:

1. Confirming the work of Holmes, there is no movement of virus from the inoculated leaf for the first 3 or 4 days. This period is slightly less or considerably more according to the greater or less activity of growth of the plant.
2. When the virus passes out from the inoculated leaf it travels first to the roots of the plant with such speed that it can seldom be intercepted at intervening positions.
3. Usually about a day later it travels with equal rapidity to the top of the plant.

4. In the earliest stages of entering the stem virus particles may be separated by considerable distances (at least several centimetres), since successive samples taken from the stem may yield lengths of 2½ cm. (the length of the cuttings) free from infection, interspersed irregularly between portions containing the infection.

5. The presence of developing fruit trusses on the stem may cause part of the virus to travel upwards as far as these trusses at the same time that the initial downward movement is occurring.

6. The virus enters developing fruits at the same time as it travels through the stem, whereas adjacent leaves remain uninfected for days or weeks.

7. In pot plants, after the initial rapid infection of the developing leaves at the top of the plant, the more mature leaves become successively invaded from the top downwards and from the bottom upwards until the plant is completely invaded by the virus. Complete invasion occurs very quickly in small vigorously growing plants: it may take 3 weeks or more in medium sized plants (Text-fig. 2); and as much as 2 months in large fruiting plants (Text-fig. 3).

8. Complete invasion never occurs when large field plants of tobacco or tomato bearing a number of mature leaves are inoculated. The mature leaves remain free from virus, apart from a limited movement along the mid-ribs, for periods of more than 3 months following inoculation (Text-fig. 4).

It is considered that these facts favour the theory of a slow cell to cell movement of the virus via the plasmodesmen, combined with a rapid distribution through the plant via the phloem, and the value of tobacco mosaic virus as an indicator of phloem movements is emphasised.

The author is indebted to Mr J. G. Bald for helpful criticism of the manuscript.

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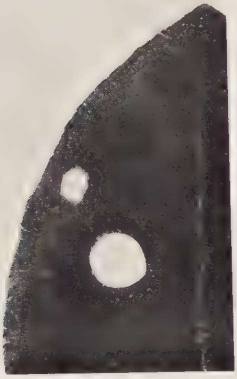

Fig. 1.

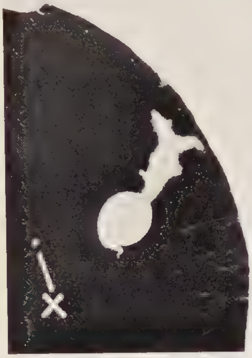

Fig. 2.

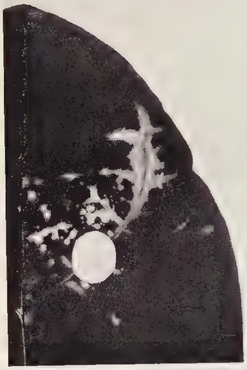

Fig. 3.

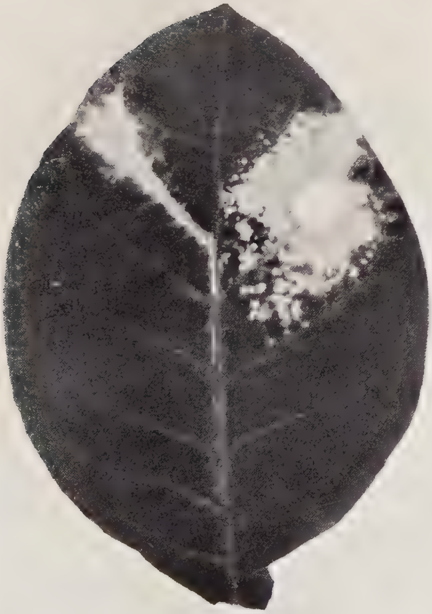

Fig. 4.

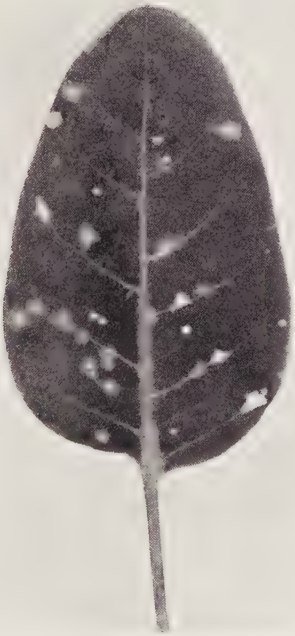

Fig. 5.

SAMUEL.—The Movement of Tobacco Mosaic Virus within the Plant (pp. 90–111).

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EXPLANATION OF PLATE IV.

Figs. 1–3. Leaves of Blue Pryor tobacco, inoculated over a lateral vein near the margin with yellow tobacco mosaic. Prior to inoculation a disc of tissue was removed with a cork borer, interrupting the lateral vein about 1 cm. below the point of inoculation. Leaves removed after various intervals, killed in steam, decolorised with alcohol, and stained with iodine. Fig. 1, 6 days after inoculation; systemic symptoms just appearing. Fig. 2, 8 days after inoculation; note the single spot of virus multiplication (opposite **X**) which has appeared in the mesophyll below the vascular interruption. Fig. 3, 11 days after inoculation.

Fig. 4. Similar to the above, but inoculated on both sides of the leaf, the lateral vein being interrupted below the point of inoculation on one side only. 14 days after inoculation.

Fig. 5. Upper leaf of a *N. glauca* plant inoculated 6 weeks previously with yellow tobacco mosaic. Leaf taken from one of the three plants, out of thirty inoculated, which showed symptoms on leaves above the inoculated leaves. The symptoms consist only of localised yellow spots, in most cases definitely associated with the veins of the leaf.

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APHIDES AS VECTORS OF "BREAKING" IN TULIPS. II.

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(With Plate V.)

Introduction.

The tulip experiments which were begun in 1928(2) at the John Innes Horticultural Institution have been continued during the following four seasons. In 1930–1 the method adopted of using aphis-proof muslin over plots of tulips in the open proved a failure, as the muslin excluded too much light so that the plants failed to flower the following season. In 1931, as a result of generous response to an appeal for funds to erect a special tulip house, the bulbs were again housed under glass, but it was not until this season that they were nursed back into flower and scored. In the succeeding account of the experiments various terms are constantly being used which it would be as well to explain here. "Full break[1]" is the term used to denote the type of infection in which the yellow or white mesophyll colour is revealed by the receding "breeder" or "self" colour. "Self break[2]" is where the "breeder" or "self" colour is intensified into darker streaks or stripes. "Clotted" or "clotting" denotes the type of infection manifested in dark purple or dark red shiny varieties which never show the ordinary bicoloured "full break," but intensify the "self" colour in great splashes and patches. "Heavy" and "light" are terms denoting intensity of infection.

The experiments of 1929–30.

The tulips, all of the variety Bartigon, were grown in the open under muslin in the season 1930–1 in order to watch the results obtained in the first year, and to ascertain whether they would alter in succeeding seasons. Unfortunately this treatment gave the plants a severe check, and it was not until the present year when they had been grown for two successive seasons under glass that the plants flowered sufficiently to allow a final scoring to take place. Many succumbed to the treatment out of doors and possibly to other causes as well, but there remained a sufficient number to draw certain tentative conclusions. It would appear that "self break" is variable in a way that is never experienced with "full break." In some instances the marking had become more pronounced than in 1931 (the last year in which scoring was possible) whereas in others the streaking was less noticeable, and in a few plants, more especially where the initial "breaking" had been very slight, the flowers had reverted to normal breeders. A "full break"—the normal "break" of florists—will vary from year to year in the extent of the white or yellow and in the pattern, but never reverts to the "breeder" state. There was still no "breaking" in the tulips which had been infested in 1929–30 with aphides from Arran Victory carrying potato mosaic.

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[1] Called "White break" in previous communications (2, 3).

[2] Called "Red break" in previous communications (2, 3).

The experiments of 1930–1.

During this season the experiments were designed to fall under three heads: (1) susceptibility of different varieties to the same source of infection; (2) susceptibility of one variety to different sources of infection; (3) attempted production of "parroting" by means of aphis infection.

All these experiments were in the open under muslin cages, and units of 50 bulbs were chosen for the treated plots. The controls, 50 in number, of each variety were "unbroken." As several experiments were undertaken with the variety Moralis, however, the number of the controls of this variety was increased to 200.

(1) *Myzus persicae* Sulz, after having been fed upon the "broken" variety Elizabeth Pegg for at least 14 days, was transferred to the following varieties: Bartigon, Rev. Ewbank, Couleur Cardinal, Prince of Austria, Philippe de Commines, Mrs Potter Palmer, Gesneriana Spathulata, Panorama, Isis, Bronze Queen and Moralis. Owing to the same causes as mentioned earlier, the plants failed to flower till this year and many were lost altogether. The results were:

Bartigon:	31 blooms, 6 "full breaks"
Couleur Cardinal:	14 blooms, no "breaks"
Prince of Austria:	31 blooms, 6 "full breaks"
Mrs Potter Palmer:	6 blooms, 1 "full break"
Gesneriana Spathulata:	8 blooms, no "breaks"
Panorama:	9 blooms, 8 "breaks"
Isis:	17 blooms, 3 "clotted"
Bronze Queen:	5 blooms, 1 "full break"
Moralis:	20 blooms, 2 "breaks"

This would seem to indicate that some varieties are more susceptible than others, but little value can be attached to the relative figures. The virus has a pronounced weakening effect upon the tulip, consequently the losses of bulbs owing to the bad environment in 1931 would fall chiefly upon the infected bulbs. It is very necessary to ascertain if the apparent immunity of certain varieties can be confirmed.

The second part of the same experiment was to ascertain if *Macrosiphum gei* Koch, alone or in conjunction with *Myzus persicae*, under similar circumstances would produce a different result. The source of infection was again Elizabeth Pegg.

With *Macrosiphum gei* alone:
  Moralis: 11 blooms, 2 "full breaks"
With *Myzus persicae* and *Macrosiphum gei*:
  Moralis: 11 blooms, 6 "full breaks" very white

(2) The variety Moralis was used as the tulip to be infected in this series of experiments. This was an unfortunate choice, as "self break" is very difficult to differentiate in this variety. The four sources of infection were Esopus, Bartigon, Sulphur and Talisman in their "broken" forms.

Myzus persicae:

From Esopus:	17 blooms, 13 "full breaks"
From Bartigon ("self breaks"):	24 blooms; several flowers were odd and may have been "self break"
From Sulphur:	28 blooms, 4 "full breaks," 1? 1 "self break"
From Talisman:	24 blooms, 1 "self break"

While this suggests that in certain varieties the virus is less virulent than in others, a more critical experimental procedure is required before a conclusion can be drawn.

(3) Moralis was again used in some of these experiments with Markgraaf van Baden as the parrot tulip for infection, and Clara Butt with its parrot Fantasy was used for the others. In no case was the parrot condition transmitted. The conclusion to be drawn from these and from the earlier experiments is that parrot is not transmissible by this means, and is in all probability a somatic sport. Parrot tulips, *e.g.* Fantasy, may become broken just as the breeder, Clara Butt, from which Fantasy originated will "break," and the breeder and "broken" forms of the same parrot variety are clearly distinguishable.

The experiments of 1931–2.

All the bulbs were planted in the new tulip house, and the work was divided into three main and six subsidiary experiments. One hundred bulbs of each of the varieties used were kept as controls and showed no "breaking."

The main experiments.

Three plots containing 100 plants of each of the following four varieties, Bartigon, Clara Butt, Prince of Austria and Zulu, were partitioned off with three-ply boarding and faced with aphis-proof muslin. Three species of aphides were used, *Myzus persicae* Sulz, *Macrosiphum gei*

Koch, and *Anuraphis tulipae* B. de Fonsc., and were introduced on "broken" Sulphur plants growing in pots. From time to time the aphides were also distributed by means of a camel-hair brush as evenly as possible over the whole plot. The aphis colonies were not satisfactory this season, partly owing to a severe attack of the Braconid parasite *Aphidius matricariae* Hal. and possibly partly owing to having been kept parthenogenetically since 1928. Very little "breaking" was seen in 1932, but in 1933 the results were as follows:

With *Myzus persicae*:
Prince of Austria: 40 "unbroken," 10 "full breaks," 5 "self breaks"
Bartigon: 37 "unbroken," 13 "full breaks"
Clara Butt: 20 "unbroken," 13 "full breaks," 1 "self break"
Zulu: 16 "unbroken," 8 "clotted," 2 doubtful

The stock of Zulu contained as rogues 16 bulbs of an unidentified tulip, four of which became "full breaks."

With *Macrosiphum gei*:
Prince of Austria: 76 "unbroken," 1 "full break," 1 self "break"
Bartigon: 72 "unbroken," 3 "full breaks"
Clara Butt: 31 "unbroken," 5 "full breaks," 1 very heavy "self break"

The fourth stock proved to be almost wholly the red tulip mentioned above. There were three Zulus which remained "unbroken." *Macrosiphum gei* suffered more from the parasite than *Myzus persicae*.

With *Anuraphis tulipae*:
Prince of Austria: 157 "unbroken," 3 "self breaks"
Bartigon: 149 "unbroken"
Clara Butt: 117 "unbroken," 2 "full breaks"
Zulu: 75 "unbroken"

One feature that was very evident in the consideration of these plots was the weaker growth where the tulips had been infected. Where *Myzus persicae* had been used as the carrier 129 tulips bloomed, with *Macrosiphum gei* 230 bloomed, while in the compartment receiving *Anuraphis tulipae* there were 498 that bloomed. Similar differences in vigour were seen in the foliage. Thus the proportion of tulips infected by *Myzus persicae* in particular must have been much greater than the figures indicate.

As in previous experiments *Myzus persicae* proved to be a vector of "breaking" in a greater degree than *Macrosiphum gei*. When applied to the growing tulip *Anuraphis tulipae* failed to transmit infection, since the few "breaks" seen may be dismissed as probably impurities in the stock.

Four of the subsidiary experiments were designed to see at what time in the development of the plants the virus of "breaking" ceases to be effective. For this purpose four plots of 100 Bartigon each were set aside for late infection, two by *Myzus persicae*, and two by *Macrosiphum gei*, both infected by feeding upon "broken" Lady Moira Cavendish. Two plots were infested on June 3rd, 1932, and two a week later. By this time the plants were beginning to dry off and no infection took place except for one bulb in the later *Macrosiphum gei* infection which was a "full break" most probably due to impurity in the stock.

The last two experiments were also each with plots of 100 Bartigon and with *Myzus persicae* as the vector. In the first the source of infection was "self-broken" Bartigon. Of the 36 tulips which bloomed all were "self breaks." In the second the source of infection was "clotted" Zulu. Of the 66 which bloomed 55 were "full breaks," 3 were "self breaks," and 8 were breeders. These two results seem to show that "self break" will only produce "self break," and that "clotted" is the expression of "full break" in those dark purple and dark red varieties with a shiny texture.

THE EXPERIMENTS OF 1932–3.

The experiments this season were fourfold: (*a*) time of infection in conjunction with depth of planting; (*b*) intensity of infection; (*c*) duration of infection; (*d*) experiment on bulbs with *Anuraphis tulipae*. All the bulbs used were the variety Bartigon and with the exception of Exp. D the aphis used was *Myzus persicae*. The 100 Bartigon controls were all normal.

Exp. A. Three plots were partitioned off in the tulip house in a similar manner to those in last season's experiments. The first contained 50 bulbs planted at 12 in. deep. This was infested by the aphides when the tulips were just coming into flower, *i.e.* the normal time for infestation to take place in the open. The second plot contained 50 bulbs planted at a depth of 6 in. and 50 at 12 in. This was infested when the first flowers were beginning to fall. The third plot was similar to the last and was infested a week later. The purpose of the experiment is to ascertain at what period the tulip ceases to be susceptible to infection, and whether those planted deeper are less subject to infection than those planted at the normal level.

Exp. B. This was with individual tulips planted in units of 20, and was divided into four sections. The technique followed was the same for each group. When the shoots were showing 3 in. above the ground each plant was covered with a hurricane lamp glass with muslin top kept in place by a rubber band. The aphides were previously allowed to infest "broken" material for at least 14 days.

(1) One aphid was placed on each plant and allowed to remain 2 days, after which the lamp glasses were removed and the aphides killed. When the flowers appeared one was a very heavy "self break" with some white spreading from the base, all the others were sound.

(2) Ten aphides were placed on the plants as before and left on for 2 days. Of the blooms in this plot three showed heavy "self break" (two with white spreading from the base), one was a half heavy "self break," that is, half the flower was heavily infected and the other half was normal, and two were light "self breaks."

(3) Fifty aphides were placed on the plants and left for 2 days. The resulting "breaks" were not significantly more numerous than those in the last plot. There were four very heavy "self breaks," one showing much white at the base, one half heavy "self break" and four light "self breaks." Two of these last developed late when they had been in full flower and normal for some time.

(4) One aphid was placed on each plant and left on for a week. The result was the same as in (1) above, only one very heavy "self break" appearing.

A second season of growth is necessary before full conclusions can be drawn, but it is very evident that "self breaking" can become manifest in the same season as the foliage is infected.

Exp. C was an endeavour to determine how long it is necessary for an aphid to feed on "broken" material in order to pick up the virus. Again the plots were of 20 bulbs, and the aphides were placed on "broken" plants for 1, 2, and 3 days respectively. The same technique with regard to hurricane lamp glasses as in Exp. B was followed, and the plants were again infested when the leaf spathes were showing 3 in. above the ground. Ten aphides were placed on each plant and allowed to remain for a week. In the plot in which the aphides had been allowed to feed only 1 day on the "broken" material, there were no "breaks." In the "two-day" plot there were two very heavy "self breaks" and one light "self break," but the offset to this plant gave a very heavy "self break." In the "three-day" plot there was one very heavy "self break" with the offset a light "self break," and one light "self break."

Again no conclusions can be drawn till next season's flowering.

Exp. D. The bulbs used in this experiment were mixed in store with "broken" bulbs infected with *Anuraphis tulipae*. The aphides soon spread throughout the whole box. The bulbs were then cleaned and planted. Of the 29 flowers, 6 were "full breaks" and 1 was a "self break." All the plants were much dwarfed, and many showed other signs of damage. *Anuraphis tulipae* must therefore be considered as a vector of "breaking" when the infestation takes place in the bulb store, though when feeding on the leaves it does not appear to transmit the virus in any marked degree.

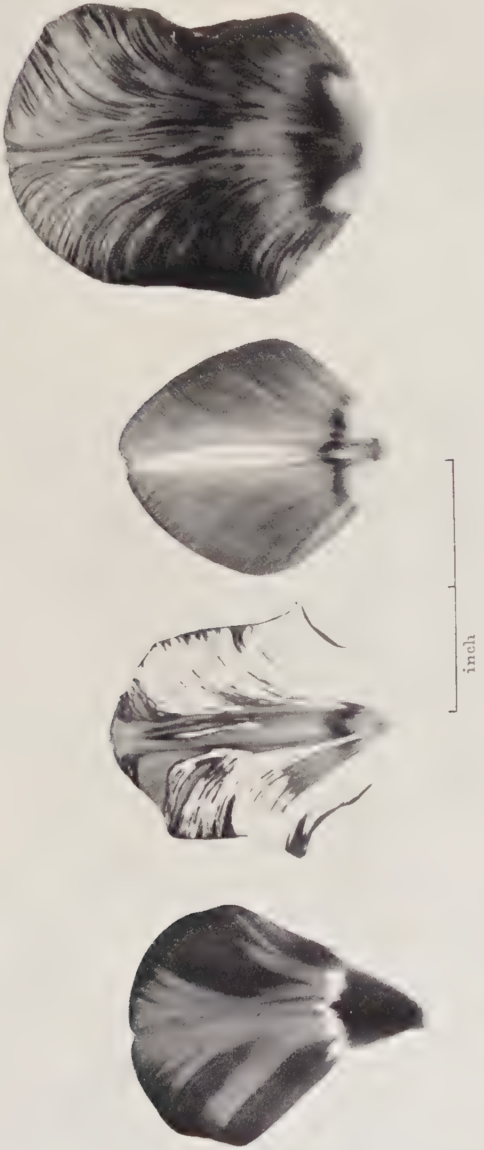
DISCUSSION.

Since the inception of these experiments Dr F. P. McWhorter (1) has stated that he has succeeded by hypodermic needle inoculations in isolating two viruses. These viruses are possibly identical with "self break" and "full break," which from the foregoing experiments must definitely be considered as different types of infection, and not as previously suggested as phases of the same virus (3). We may provisionally conclude that two viruses are present in the ordinary "full break." The evidence is clear that the virus of "self break" may be selected by the vector from a "full break" tulip but that a "self break" tulip can then only transmit "self break." In these experiments there has been no definite evidence of a transmission of the second virus alone. "Full break," as has been stated above, is comparatively constant, but "self break" is not so stable, as in some instances it has reverted to normal. There is some evidence to show that certain varieties are more susceptible to attack, and that some also carry a more virulent form of virus than others.

Parroting is not transmissible by means of aphid vectors, and it seems more probable that it is a "sport" than a virus infection.

"Clotting" appears to be the manifestation of "full break" in the dark purple and dark red varieties which possess a shiny texture to the petals, and not of "self break" as might have been expected, though it will be necessary to suspend judgment till next year's results are known.

Definite evidence has been obtained that the plant becomes immune from virus infection at a certain stage. Presumably this is correlated with the time at which the new bulbs and offsets become cut off by dead tissue from the still growing and flowering stem which perishes with the season of flowering. In the garden, instances are occasionally seen of what was planted as a single breeder bulb producing its main flower "broken," but also an offset with a breeder flower, or *vice versa*. It was formerly supposed that the early tulips, varieties or species, did not "break," whereas after experimental infection they prove to "break" just as readily as the May flowering garden tulips. It may be concluded that these early tulips have passed the stage of susceptibility before the aphis normally reaches the garden.



McKENNY HUGHES.—Aphides as Vectors of "Breaking" in Tulips (pp. 112–119).

There are indications that, except for the mechanical damage they do in weakening the plant, a large number of aphides do not necessarily carry a proportionately larger amount of virus than a small number, and it would also appear that more than 24 hours must elapse before an aphid can pick up the virus from an infected plant.

Anuraphis tulipae B. de Fonsc. is frequently found in the bulb store, and is a definite vector of "breaking" when attacking the bulbs but not when feeding upon the foliage.

Summary.

1. Three types of "breaking" are distinguished—"full," "self" and "clotted."

2. The evidence points to "full break" being the product of two viruses.

3. "Self breaking" may be selectively transmitted by the aphis vectors *Myzus persicae* Sulz and *Macrosiphum gei* Koch.

4. "Self break" tulips only transmit "self breaking."

5. "Clotting" is an expression of "full break" in certain varieties.

6. *Anuraphis tulipae* B. de Fonsc. is a definite vector in the bulb store but not on the growing plant.

7. At a certain stage of growth tulips cease to be susceptible to infection.

Acknowledgment.

Thanks are again due to Sir Daniel Hall for his help and advice throughout the experiments.

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(2) McKenny Hughes, A. W. (1930). Aphis as a possible vector of "breaking" in tulip species. *Ann. App. Biol.* xvii, 36–42.

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EXPLANATION OF PLATE V.

Left to right:
Fig. 1. "Clotted break" (Isis).
Fig. 2. "Full break" (Bartigon).
Fig. 3. "Self break" (Bartigon), light.
Fig. 4. "Self break" (Bartigon), heavy.

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SOME COMMON SPECIES OF THE GENUS *THRIPS* (THYSANOPTERA)

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(With Plates VI and VII and 9 Text-figures.)

I. Introduction.

The Thysanoptera constitute an order of the class Insecta, and are commonly known by the name "Thrips," given by Linnaeus as a generic title to the species *Thrips physapus* which inhabits the flowers of dandelion. The relation of this order to others of the class has remained uncertain: usually an alliance with the Hemiptera is indicated, but there are also affinities with the Orthoptera, the Zoraptera perhaps forming a connecting link between the two. "Thrips" are small slender insects, attaining a length of at most one-third of an inch, the forms usually met with seldom exceeding one-twentieth of an inch. Associated generally with the flowers or foliage of plants, these insects have of late attracted attention as being vectors of certain types of mosaic disease in plants. They are of world-wide distribution, and those of tropical or sub-tropical origin are often introduced into hot-houses in temperate climates, and become established in them as pests of rare and valuable plants. Though such forms do not appear to be able to adapt themselves to outdoor conditions of colder climates, the Onion Thrips, *T. tabaci* Lind., occurs plentifully in both temperate and tropical zones. Not a few are well known as serious pests of tropical agriculture, while others are destructive to the blossom of fruit trees, to fodder crops, to horticultural crops, including those grown under glass, and to flowers grown for the market.

It is intended to unravel some of the confusion which has arisen in the past from the inadequacy of the descriptions given for the imagines of some extremely common, and in some cases very injurious, species of the genus *Thrips*: the young stages cannot at present be dealt with, but fortunately the adult insects are almost always obtainable when the development of their offspring is proceeding.

The material here set out may perhaps indicate lines upon which future investigations might be pursued in order that a revised classification of the

Thysanoptera, so urgently needed, may be constructed upon a basis which has practical value as well for the systematist as for the biologist.

II. CONFUSION BETWEEN THE SPECIES *T. TABACI* LIND., *T. FUSCIPENNIS* HAL., AND *T. MAJOR* UZEL.

(Plates VI and VII.)

During a study of two destructive species of Thrips, namely the Onion Thrips (*T. tabaci* Lind.) and the Rose Thrips (*T. fuscipennis* Hal.), which occur in greenhouses as pests both of the cucumber plant and of roses, difficulties were encountered in making identifications of the specimens obtained. Identified according to the descriptions given by H. Priesner in his monograph *Die Thysanopteren Europas* (Vienna, 1926–8), males of *T. tabaci* Lind. always occurred with females of *T. fuscipennis* Hal., while females of *T. tabaci* Lind. always bred parthenogenetically, males being entirely absent. When collections were made from flowers of Arum, moved out-of-doors in June, males and females of *T. tabaci* Lind. were abundant, together with comparatively few males of *T. fuscipennis* Hal., and extremely few females of the latter species and its varieties *major* (Uzel) and *banaticus* (Knechtel). In flowers of outdoor roses and white convolvulus during the summer months, females of *T. tabaci* Lind. were absent, while males of the latter species together with males and females of *T. fuscipennis* Hal. with its varieties were present in enormous numbers. It therefore appeared probable that Priesner had assigned the wrong males to their respective females, and that he had made an error in treating Uzel's species *T. major* as a variety of *T. fuscipennis* Hal. Conclusive proof of this has now been obtained.

Dr Guy Morison has collected males of *T. tabaci* Lind. in Scotland: these differ entirely from the description for the male of this species given by Priesner, and from the males of other common species of the genus *Thrips*. They have, however, important structural characters in common with the females with which they were collected, and these are undoubtedly referable to the species *T. tabaci* Lind. Their discovery is of importance in showing that the Onion Thrips is not always an entirely parthenogenetic species.

At the Cheshunt Experimental Station, during the spring and summer of 1932, males of *T. fuscipennis* Hal. were obtained by breeding from females of that species under completely isolated conditions. These males agree with Priesner's description of the male of *T. tabaci* Lind. A third type of male, agreeing with Priesner's description of *T. fuscipennis*, male, has been found to belong to the species *T. major* Uzel, wrongly considered by Priesner to be a variety of *T. fuscipennis* Hal. *T. banaticus* Knechtel is synonymous with *T. major* Uzel, as an examination of numerous specimens shows every gradation between the two females, and there is only one type of male.

The confusion amongst these species therefore resolves itself as follows:

Priesner's *T. tabaci* Lindeman, male = *T. fuscipennis* Haliday, male.

Preisner's *T. fuscipennis* Haliday, male = *T. major* Uzel, male.

Priesner's *T. fuscipennis*, formae *major* (Uzel) and *banaticus* (Knechtel) = *T. major* Uzel, female.

T. tabaci Lindeman, male, has hitherto not been described.

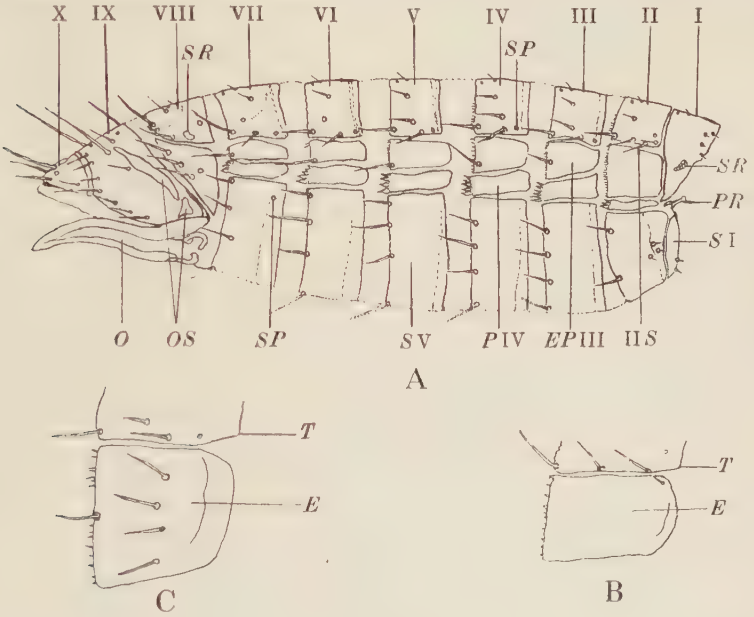
It still remained to find some constant characters by which the females of *T. fuscipennis* Hal. and *T. major* Uzel might be separated from one another. Superficially the two are extremely alike, although their respective males can be distinguished almost at a glance. An attempt was made to separate the two females upon characters largely used by systematists for other species of the genus, namely the dimensions of the several antennal joints and those of the maxillary palps, the length of the setae upon the head, prothorax, ninth, and tenth abdominal tergites, the colour of the fore-wings, and the number of setae upon their veins. Of these, only the colour of the fore-wings, the number of setae upon the costal margin, and the length of the fourth and fifth antennal joints, gave any indication of difference between the two species. But when a series of specimens was divided into two groups upon any one of these characters, there was always overlapping in all the other characters mentioned. The colour of the fore-wings, dusky in the one, and light yellow in the other, was the most constant, but was not applicable to specimens lately emerged from the pupal stage.

Finally, differences in the number of setae present upon the side margins of the second abdominal tergite and upon the first abdominal sternite gave obviously constant characters by which the two could with absolute certainty be distinguished.

T. fuscipennis Hal. (Text-figs. 1 A and 2 B). Along the outer (side) margins of the second abdominal tergite, on each side of the segment, a longitudinal row of *four* setae (with an anteriorly placed seta-pit). Upon the anterior margin of the first abdominal sternite, two very small but conspicuous setae, one on either side of the middle line.

T. major Uzel (Text-fig. 2 C). Along the outer (side) margins of the second abdominal tergite, on each side of the segment, a longitudinal row of *three* setae, with an anteriorly placed seta-pit. The first abdominal sternite devoid of setae.

These characters apply also to the male sex (Text-figs. 2 B and 2 C), but here the difference in arrangement of the setae upon the ninth abdominal tergite is more easily seen (Text-fig. 3). The females of *T. fuscipennis* Hal. and *T. major* Uzel were then found to vary in the less reliable characters as follows:



Text-fig. 1.

A. *T. fuscipennis* Hal. Female. Abdomen viewed from right side. × 100. I–X, tergites. II *S*, lateral margin of second tergite, with four setae. *EP* III, epipleurite of third segment. *O*, ovipositor. *OS*, sclerites of ovipositor. *P* IV, pleurite of fourth segment. *PR*, rudimentary pleurite of first segment. *S* I, sternite of first segment. *S* V, sternite of fifth segment. *SP*, seta-pit. *SR*, spiracle.

B. *T. physapus* L. Female. Portion of second abdominal segment, viewed from right side. × 230. *E*, epipleurite with one seta. *T*, lateral margin of tergite with three setae and seta-pit.

C. *T. minutissimus* L. Female. Portion of third abdominal segment, viewed from right side. × 230. *E*, epipleurite with four intermarginal setae. *T*, lateral margin of tergite with three setae and seta-pit.

	No. of specimens	Antenna		Costal setae	Specimens with	
		4th joint	5th joint		Dusky wings	Light wings
T. fuscipennis Hal.	125	35–43 μ	30–38 μ	23–29	123	2
T. major Uzel	50	41–53 μ	34–43 μ	24–33	0	50

The finding of reliable structural differences naturally led to a study, in other species, of the abdominal chaetotaxy, hitherto almost entirely neglected in descriptions of species, genera, or higher divisions of the Thysanoptera, and valuable characters by which species may readily be identified have come to light, especially in relation to the arrangement of setae upon the sternites and epipleurites and the second tergite of the abdomen. These are fully set out later.

III. VARIATION IN THE ANTENNA AND WING.

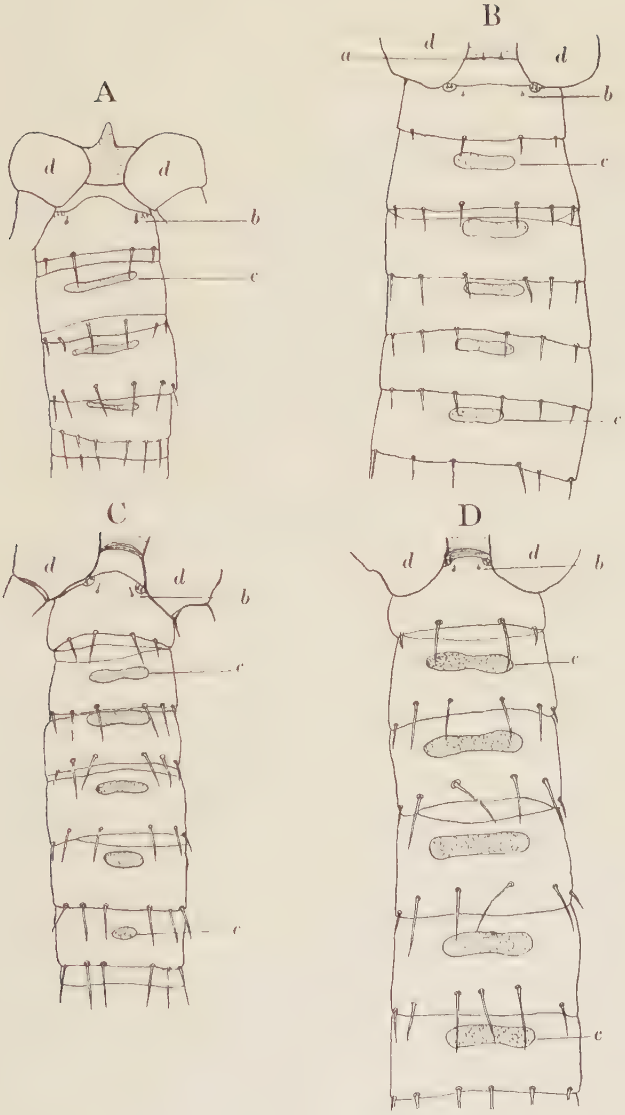
The naming and separation of species at least within the genus *Thrips* upon characters of the antennae, both as regards colour and dimensions of the several joints, have undoubtedly led to the greater part of the confusion which at present exists.

The average length measurements of the fourth and fifth antennal joints of females of *T. fuscipennis* Hal., collected from different plants in different environments, tend to show that environment may be a factor which influences the length of these joints:

T. fuscipennis Hal. Antenna.

Origin of specimens	No. of specimens	Average length	
		4th joint	5th joint
Rose under glass	37	40·52 μ	35·77 μ
Rose out-of-doors	24	40·08 μ	34·96 μ
Cucumber under glass	33	39·15 μ	34·97 μ
Various flowers out-of-doors	31	39·74 μ	35·26 μ
Total	125	Av. 39·88 μ	35·28 μ

The average length of these joints in 50 females of *T. major* Uzel was 43·49 μ and 38·52 μ respectively; the higher average does not constitute a specific character, as some specimens fall within the range of variation exhibited by *T. fuscipennis* Hal. and *vice versa*. A similar overlapping occurs in the sixth antennal joint of *T. physapus* L. and *T. validus* Uzel, the females of which again are superficially very similar, while the males are easily separable from the depressions upon the third to seventh abdominal sternites, which are very large in the former, and very small in the latter species. Both species inhabit the flowers of dandelion, generally in each others' company. The sixth antennal joint of *T. physapus* L., female, measures 51–59 μ in length, while that of *T. validus* Uzel measures 45–52 μ. The colour of this joint is usually dark in the latter species, but in some specimens it is light in the proximal half, as in *T. physapus* L., this applying to both sexes. Hitherto the females of



Text-fig. 2. Abdominal sternites of males. ×175. A, *T. tabaci* Lind., sternites 1–5. B, *T. fuscipennis* Hal., sternites 1–7. C, *T. major* Uzel, sternites 1–7. D, *T. flavus* Schrank, sternites 1–7. *a*, minute setae on first sternite. *b*, minute setae on second sternite. *c*, glandular depressions. *d*, coxae of posterior legs.

these two species have been separated upon the differences in the shape of the head, but in mounted specimens, at any rate, this character is unreliable.

The arrangement of setae upon the abdomen again gives constant characters by which the two species may with certainty be distinguished:

T. physapus L. Across the surfaces of each of the third to seventh abdominal *sternites* inclusive, an irregular row of conspicuous setae between the regular rows situated at the hind margin of each sternite. The former setae are termed "intermarginal" (see Text-fig. 5). Upon each *epipleurite* of the second abdominal segment, and situated anteriorly near the margin adjoining the tergite, a short seta (Text-fig. 1 B).

T. validus Uzel. The third to seventh abdominal sternites inclusive, without intermarginal setae. The epipleurites of the second abdominal segment devoid of setae.

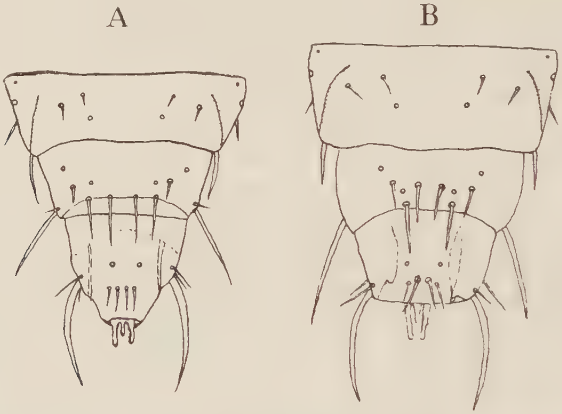
Amongst a number of species of the genus *Thrips* described by Priesner, *T. praetermissus*, *T. montivagus* and *T. pillichi* deserve mention in relation to the antennal characters. Priesner states that these species very closely resemble *T. tabaci* Lind. (or *T. flavus* Schrank). From co-types and para-types now in the British Museum and from fresh specimens collected from the flowers of dandelion and yarrow, it is at once apparent that they differ from the latter species very obviously in the colour of the ocellar pigment. This pigment is bright red or orange in the majority of species of the genus *Thrips* (and in many other Thysanoptera), but in the species *T. tabaci* Lind. and *T. juniperina* Bagnall it is grey. Priesner separates the species in question from one another and from *T. tabaci* Lind. largely upon the proportional length of the sixth to the seventh antennal joint. Measurements of the sixth and seventh antennal joints in a long series of *T. tabaci* Lind., female, show that the former may be from 2·2 to 3·2 times the length of the latter, and in the male as much as 3·5 times. It transpires then that Priesner's three species are all one and the same, and that he has merely recorded the variation in the proportional length of the two distal antennal joints in describing them under three names. The name *T. praetermissus* Pr. holds priority, and this species can be separated from *T. tabaci* Lind. at a glance by its possession of red (instead of grey) pigmented crescents partially surrounding the ocelli, and of intermarginal setae upon the abdominal epipleurites and sternites, which are entirely absent in *T. tabaci*. In these respects *T. praetermissus* Pr. resembles *T. minutissimus* L. (Text-fig. 1 C), from which it is, however, distinct.

Temperature appears to have some influence upon the colour of the fore-wings in mature imagines of *T. fuscipennis* Hal. Females, which have over-wintered in greenhouses and have thus been unexposed to low temperatures, have the fore-wings of the normal dusky grey colour, while in those which have hibernated out-of-doors, the fore-wings are of a yellowish brown tint. The number and arrangement of the comparatively short and stout setae, known as "macrotrichia," upon the veins of the fore-wings have attracted the attention of systematists as a help to the separation of species (see Text-fig. 4). Along the costal margin, these setae are arranged at fairly regular intervals, and their number appears to vary proportionately to the length of the wing: as this length is to a considerable degree variable in individuals of the same species, the number of setae upon the costal margin cannot be taken as a reliable character. There is still greater variation in the number of setae upon the hind-vein. Probably in all species of the genus *Thrips*, the setae of the fore-vein are placed in two groups of four and three, from the wing base towards the proximal third of the wing length, with a gap between them: one or other of these groups may have a slightly greater or less number in individuals of the same species. At long intervals in the distal two-thirds of the wing length, there are usually three setae, but within the species there may be a variation of from two to five. In certain species, however, the macrotrichia in this position appear in larger numbers and at regular intervals, but it is doubtful if this character is of any true diagnostic value. When brachypterous and apterous forms are taken into consideration, it is clear that the chaetotaxy of the wings is valueless for the separation of species. It must be added that the number of setae upon the veins of the fore-wing of one side is seldom the same as that of the other in a single specimen. The males of most, if not all, species have rather fewer setae upon the costal and hind-veins than their respective females.

IV. VARIATION IN THE LENGTH OF SETAE.

The species of the genus *Thrips*, as at present constituted, possess, upon the posterior margin of the prothorax and situated laterally on each side, two setae which are conspicuously longer than all other setae standing upon the dorsal surface of that segment (see Text-figs. 4 and 9). Actual measurements of these have been utilised to some extent in the separation of species. While in a few instances these setae are comparatively short and stout, and in others exceptionally long, they lie between these two categories in the majority of species. The amount of variation in a single species causes constant overlapping in the length of these setae with others which stand in a neighbouring category. There appears to be some correlation in the female sex only between the length of these prothoracic setae and of those upon the ninth and tenth abdominal tergites, the latter again being subject to similar variation. As, in addition, it is seldom possible to make accurate measurements owing to the setae not being parallel to the field of vision, such measurements are in all cases to be avoided in the separation of species.

In descriptions, it is unnecessary to add to the statement that setae according to the species dealt with are short, of moderate length, long, or very long, terms which amply suffice in many instances, as valuable and constant adjuncts in the separation of species.



Text-fig. 3. Dorsal aspect of 8th, 9th and 10th abdominal segments. A. *T. fuscipennis* Hal. Male. B. *T. major* Uzel. Male. × 230.

V. Variation in colour.

Viewed by reflected light, the predominant colours of the main body divisions and legs are yellow, greyish yellow, and from yellowish or orange-brown to dark brown and black. Depth of colour depends largely upon the degree of sclerotisation, this being extremely variable within the species, and not necessarily dependent upon the time which has elapsed following emergence of the imago from the pupal stage. Much of the mature coloration however appears a few hours after this takes place. Males are usually, but not always, much lighter in colour than their respective females. The colour of the several antennal joints has been largely and often minutely recorded in the description of species. Owing to variability in shading, especially of the distal five joints, no reliance can be placed upon differences between the females of various species. The males are far more constant in this respect, and when specimens in which it is difficult to make out the chaetotaxy of the abdomen are involved the colours of the several antennal joints are of great value for purposes of identification. Whereas in the majority of species, both of the genus *Thrips* and of others, the ocelli are partially surrounded by crescents of orange, red, or brown pigment, in a few species this pigment is light grey. The ocellar colouring is a constant character, unfortunately unrecorded in many descriptions, and is similar in both sexes of the same species.

VI. SHAPE OF THE HEAD AND ANTENNAL JOINTS.

At one period a genus *Bagnallia* was raised to include species of the genus *Thrips* in which the breadth of the head was less, equal to, or only slightly greater than, its length. This genus is at present not recognised. Although it is possible that the dimensions of the head may present constant characters of specific value, it is only with great difficulty that distortion can be avoided in the process of mounting. Precautions in the mounting of type and co-type specimens have seldom been taken, so that in many the natural proportions have been entirely lost. In a large series of fairly well-mounted specimens of *T. fuscipennis* Hal., the breadth of the head has been found to vary from 1·2 to 1·6 times its length, and it becomes doubtful if this large variation is not a natural one. At all events, this character does not appear to be of practical value in the determination of species. In a few species of the genus, certain of the antennal joints diverge considerably from the usual filiform type. In these cases it appears justifiable to make use of their shape as a specific character. In general, however, a joint which is shorter than the average for a given species is also more rounded, so that no reliance can be placed upon smaller differences of shape and dimension. Unless dissections are made, it is rarely possible to obtain accurate measurements of the three proximal antennal joints, so that the comparative proportions of the third to the fifth joint cannot be readily obtained. Not infrequently, the antennae of one or both sides of a specimen are deformed, and with few if any exceptions, it is inadvisable to utilise at all the shape or proportions of the antennal joints as diagnostic characters.

VII. CHARACTERS OF VALUE IN THE DETERMINATION OF SPECIES.

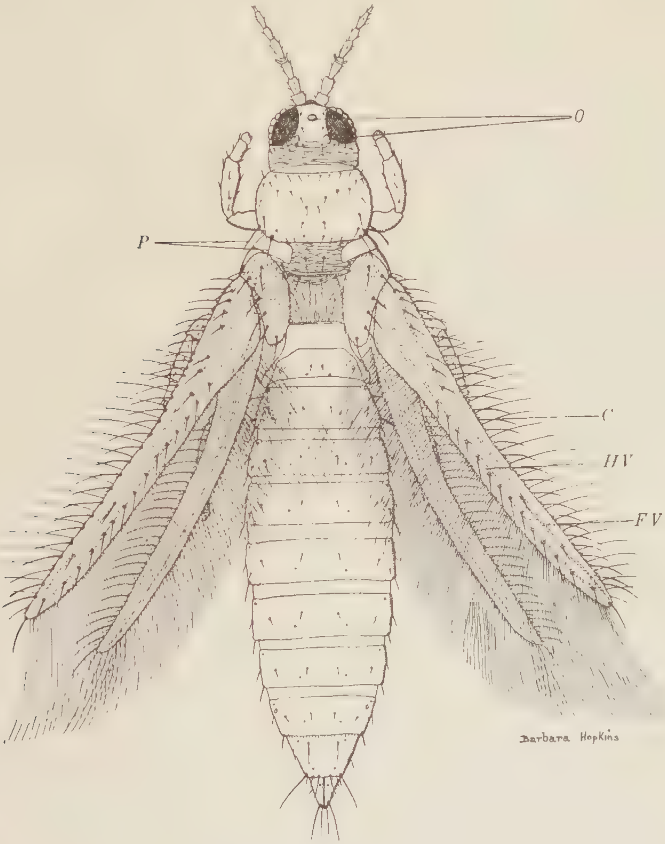
The older systematists, notably Linnaeus and Haliday, confined their descriptions of species almost entirely to the coloration of the main divisions of the body, and the general colour of the antennae, legs, and forewings. With the indifferent facilities at their disposal for the examination of such minute insects, the precision with which their work was carried out is quite remarkable.

In 1895 Uzel, in the first monograph of the Thysanoptera, added much detail and included references to the chaetotaxy, especially of the wings, in dealing with a far greater number of genera and species. Considering again that he concerned himself largely with highly variable characters, his accuracy in recognising distinct species was notable, though it is not to be wondered at that some confusion already made its appearance.

Starting with Hinds in 1902, and culminating in the works of Bagnall and Priesner, modern systematists have tended to rely, for the separation of species at least within the genus *Thrips*, upon detail of colouring and comparative measurements both of the several antennal joints and of setae upon the dorsal surface of the body, characters which have been shown above to be variable outside the limits of a single species.

In order that the more constant characters may be illustrated, it is necessary to give some description of the abdominal structure, which will, in the main, apply to a number of other genera of the sub-order Terebrantia.

The abdomen (see Text-fig. 1 A) is made up of eleven segments, the last of which is rudimentary. In both sexes the second to seventh segments inclusive are constructed similarly: in the male sex the ventral portion of the eighth segment is similar in form to the one preceding it, but in the female the sternite of this segment is profoundly modified in connection with the basal portion of the ovipositor. The arrangement of setae upon the ninth and tenth tergites is always dissimilar in the two sexes (see Text-figs. 3 and 8), and ventrally these segments are modified in conjunction with the genital apparatus. In the male sex there is, upon each of the third, fourth and fifth, and usually also upon the sixth and seventh sternites a rounded or dumb-bell shaped depression, which appears as a sharply outlined light area (Text-figs. 2 and 7); the porous chitin of these areas covers a glandular structure, the function of which may be to exude a secretion by means of which the female can recognise the male at the time of pairing. Owing to the habit of many different species often collecting together in a single flower, such a form of recognition would undoubtedly be of advantage. In both sexes, between the tergites and sternites of each of the second to seventh segments inclusive, representing the lateral portions of the segments, two distinct and separate chitinous plates are interposed. The plate adjoining the tergite of each segment is termed the "*epipleurite*," that adjoining the sternite of each, the "*pleurite*." When the abdomen is contracted the pleurites lie with their broad surfaces in a horizontal position, at right angles to the epipleurites, and often only become clearly visible in preparations under pressure. The first segment is rather differently constructed to those succeeding it. The well-developed tergite bears a prominent pair of spiracles situated laterally, the epipleurites are entirely wanting, the pleurites vestigial, and the sternite is much reduced. The anterior margin of the latter lies between the coxae of the posterior pair of legs. The epipleurites of the second segment are much broader than those of the segments posterior to it, the pleurite of this segment being proportionally narrower.



Text-fig. 4. *T. physapus* L. Female. Dorsal view. × 75. *C*, costa; *FV*, fore-vein; *HV*, hind-vein; *O*, ocelli; *P*, long setae at posterior lateral margin of prothorax.

The pleurites of segments 3-7 inclusive in both sexes are uniform, within small limits, in structure: their hind margins are produced posteriorly into a row of pointed spine-like projections, and they are always devoid of setae. Upon the lateral margins of the tergite of the eighth segment is a spiracle on each side. The general arrangement of setae and seta-pits upon the abdominal segments is illustrated in Text-fig. 1 A, while differences between the sexes in the chaetotaxy of the ninth tergite are shown in Text-figs. 3 and 8. The fundamental chaetotaxy of the abdomen is remarkably uniform, often to the minutest detail, throughout the genus *Thrips* and this uniformity extends at least to several other genera of the sub-order Terebrantia. Duplication of setae or seta-pits or the presence of an asymmetrically placed seta are of rare occurrence and are recognisable as accidental. Such divergence from the prevailing arrangement of setae and seta-pits as occurs is probably without exception a clear indication of specific difference.

Amongst those species of the genus *Thrips* which it has been possible to examine, the divergence relates, *in both sexes*, to

(1) The number and arrangement of minute setae upon or contiguous to the anterior margin of the first abdominal sternite.

(2) The number of setae upon the extreme lateral margins of the second abdominal tergite.

(3) The presence or absence of prominent setae upon the epipleurites of the second abdominal segment.

(4) The presence or absence of *additional* setae of considerable length upon the epipleurites of abdominal segments 3–7 inclusive.

(5) The presence of irregular rows of *additional* long setae upon the sternites of each of the second to seventh abdominal segments, these setae being named "intermarginal" (a term also applicable to additional setae upon the epipleurites), the absence of these from the second, or their entire absence from all the sternites.

(6) The presence of both an anterior and a posterior pair of seta-pits upon the ninth abdominal tergite, or the absence of the anterior pair.

For the male sex only:

(7) The numbers of the abdominal sternites upon which translucent depressions occur, and their approximate relative shape and size.

(8) The arrangement of setae upon the dorsal area of the ninth abdominal tergite.

For the female sex only:

(9) The character of the posterior margin of the eighth abdominal tergite, and of a transverse row of fine setae upon it, or the entire absence of the latter.

For divisions of the body other than the abdomen, absolute reliance for constancy can only be placed, in both sexes, upon

(10) The colour of the pigmented crescents partially surrounding the ocelli. This is either some shade of red or grey: in the latter case the crescents are practically invisible when viewed by reflected light.

(11) The presence or absence of hook-like processes upon the tarsal joints of the prothoracic legs. (At present only known to occur in *T. calcaratus* Uzel.)

Other characters of a much less reliable nature, owing to variation within the species, but which may be useful for purposes of identification are:

(12) The approximate relative length of the two prominent setae at the posterior lateral margins of the prothorax.

(13) The colour of the several antennal joints in the male sex only.

De Gryze and Treherne (1924) have pointed to the probable importance of the male genitalia in separating different genera from one another. The component parts of the complex armature are interpreted only with considerable difficulty, and could only be employed practically for purposes of classification when differences in their structure are very great. For the separation of species within a genus, differences, if any, could hardly be used to any advantage.

VIII. THE COLLECTION AND PRESERVATION OF SPECIMENS.

Most Thysanoptera are particularly adept in making their escape rapidly through the smallest spaces if enclosed in boxes which are not absolutely airtight. Uzel has recommended paper bags as the most suitable form of receptacle for living specimens: into these the foliage or flowers tenanted by the insects should be cut, and the bags securely fastened immediately. In the laboratory, the contents should be shaken over a large sheet of white paper, and the insects caught upon a fine moist brush before removal to a mounting medium. The practice of watching flowers in which Thysanoptera are known to occur during periods of sunshine is valuable for collecting males and females *in coitu*. Such specimens can be removed to a drop of Faure's mounting medium upon a microscope slide by means of a fine needle previously dipped in that fluid. A few specimens collected in this way are of far greater importance than many hundreds obtained haphazard. Priesner recommends the preservation of specimens in 95 per cent. alcohol, and subsequent mounting in Canada balsam. This does not appear to be a convenient method, owing to the difficulty of spreading the wings. Faure's fluid, made up as follows, has proved, from all points of view, to be a most satisfactory mounting medium for Thysanoptera in all stages of development:

Gum acacia	30 gr.
Chloral hydrate	50 gr.
Glycerine	20 c.c.
Distilled water	50 c.c.

Care should be taken that the water content is kept up to standard. If large numbers of specimens require immediate preservation, they may be placed in a weak solution of the medium, in which they will keep (sometimes alive) for at least a few days. In mounting, specimens may be arranged in two or three rows (not more than five in a row) upon a cover-glass not less than $2 \times \frac{3}{4}$ in. upon which a large drop of the medium has been placed. With a fine needle the wings and legs are spread out to a sufficient extent, and any air bubbles removed from the wings and body surface. If required, measurements of the main body divisions should be taken prior to placing a cover-slip upon the preparation. A cover-glass $\frac{1}{2}$ in. square is now gently lowered from the posterior extremities of the specimens towards their heads. Specimens mounted upon their sides are of great value, as the abdominal epipleurites, pleurites, and the lateral margins of the tergites, as well as a considerable portion of the sternites are easily examined when the insects are in this position, but such mounts are made with difficulty. In immature or in the lighter coloured species these parts can be seen in specimens mounted dorso-ventrally, and the characters of the first abdominal sternite are more easily examined: males at any rate are best mounted in this position owing to the importance of the translucent sternal depressions. Mounting between two cover-glasses enables both surfaces of a single specimen to be examined under high magnification, though in good dorso-ventral preparations the lower surface may usually be brought into focus through that lying nearest to the objective. The more-darkly coloured species often require immersion for 24 hours in 2 per cent. potassium hydrate and in some cases boiling for a short time in a 5 per cent. solution of the latter before a satisfactory examination is possible. These must be thoroughly washed in distilled water before mounting in Faure's medium. The colour of various parts of the insect are partially or wholly destroyed by potash, but observation of colour, which can always be recorded prior to treatment with potash, should always give way to that of structure.

IX. DIAGNOSTIC CHARACTERS OF SOME COMMON SPECIES OF THE GENUS *THRIPS*.

For a satisfactory examination of species of the genus *Thrips* a magnification of some 350 diameters is necessary, but for any observations relating to colour, which must without exception be viewed by reflected and diffused light, best obtained with daylight by placing the mount upon a piece of white paper and cutting off all light from beneath the microscope stage, a magnification of about 100 diameters is most convenient.

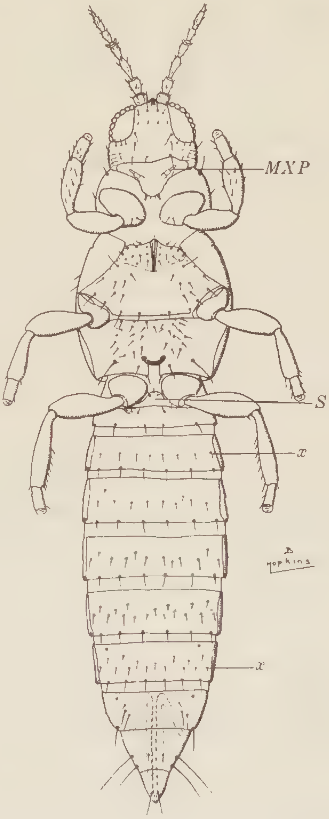
A specimen belonging to the genus *Thrips*, as at present constituted, is recognised by the following characters:

(1) Antenna composed of seven joints; maxillary palps of three joints (see Text-fig. 5).

(2) Prothorax with two setae on each side, placed laterally at the posterior margin, of much greater length than all other setae upon the pronotum (see Text-figs. 4 and 6).

(3) Tibiae of the prothoracic legs without projections, long, stout, tooth-like or hair-like setae (see Text-figs. 4–7).

These characters are extremely unsatisfactory, as they serve to distinguish this genus from certain others only upon the number of antennal joints. It is, indeed, not improbable that specimens of a single species have been placed in two separate genera owing to the distal joint of the antenna being divided in some individuals and not in others. Until fuller descriptions of the structural characteristics of the species upon which each genus is founded are available, it will not be possible to remedy this anomaly.



Text-fig. 5. *T. physapus* L. Female. Ventral view. × 75. *MXP*, maxillary palp; *St* I, first abdominal sternite; *x*, intermarginal setae on abdominal sternites 3–7.

It is proposed first to give the distinctive characters of six very common British species, two on account of their economic importance, one as being the type of the genus, and the remaining three as being, in the female sex, superficially resemblant to one or other of the foregoing species, but easily distinguishable upon structural differences explained previously.

T. tabaci Lindeman.

Female (Plate VII, fig. 5.)

Length about 1 mm. General colour light greyish yellow to brown.

(*a*) Ocellar crescents grey or light brown, almost invisible when viewed by reflected light.

(*b*) Setae at lateral posterior margin of prothorax short and stout (Text-fig. 9 A).

(*c*) Along extreme lateral margins of second abdominal tergite, three setae (with an anteriorly placed seta-pit) (cf. Text-fig. 1 B).

(*d*) Posterior margin of eighth abdominal tergite straight, beset with a complete fringe of fine setae arranged close together and at regular intervals (Text-fig. 8 A).

(*e*) Ninth abdominal tergite with only one (posterior) pair of seta-pits (Text-fig. 8 A).

(*f*) First abdominal sternite devoid of setae (cf. Text-fig. 2 A).

(*g*) Sternites and epipleurites of second to seventh abdominal segments, inclusive, devoid of intermarginal setae (cf. Text-figs. 1 A and 2 A).

Male (Plate VI, fig. 1).

Length about 0·8 mm. General colour light yellow. Characters (*a*), (*b*), (*c*), (*e*), (*f*) and (*g*) as in female. Ninth abdominal tergite dorsally with one pair of anterior setae, and four posterior setae arranged in a transverse row (cf. Text-fig. 3 A). Translucent depressions present only upon the third to fifth abdominal sternites inclusive: transversely very long; extremely narrow (Text-fig. 2 A). Colour of antennal joints: (1) Colourless. (2) Yellow. (3) Peduncle colourless with a narrow brownish yellow transverse band at its apex. Proximal three-quarters yellow, distal quarter brownish yellow, or unicolorous yellow. (4) Proximal two-thirds light yellow, distal third brownish yellow. (5) Proximal half yellow, distal half yellowish brown. (6) Proximal third yellow, distal two-thirds yellowish brown. (7) Yellowish brown.

T. flavus Schrank.

Female (Plate VII, fig. 8).

Length about 1·3 mm. General colour light yellow to orange yellow.

(*a*) Ocellar crescents orange.

(*b*) Setae at lateral posterior margin of prothorax very long (Text-fig. 9 C).

(*c*) Along extreme lateral margins of second abdominal tergite, four setae (with an anteriorly placed seta-pit) (cf. Text-fig. 1 A).

(*d*) Posterior margin of eighth abdominal tergite straight, beset with a complete fringe of fine setae arranged close together and at regular intervals (Text-fig. 8 C).

(*e*) Ninth abdominal tergite with an anterior and a posterior pair of seta-pits (Text-fig. 8 C).

(*f*) First abdominal sternite devoid of setae (cf. Text-fig. 2 D).

(*g*) Sternites and epipleurites of second to seventh abdominal segments, inclusive, devoid of intermarginal setae (cf. Text-fig. 1 A).

Male (Plate VI, fig. 4).

Length about 1·0 mm. General colour yellow. Characters (*a*), (*b*), (*c*), (*e*), (*f*) and (*g*) as in female. Ninth abdominal tergite dorsally with one pair of anterior setae, and four posterior setae arranged in a transverse row (cf. Text-fig. 3 A). Translucent depressions present upon the third to seventh abdominal sternites inclusive: large and somewhat elongate transversely (Text-fig. 2 D). Colour of antennal joints: (1) Colourless. (2) Colourless. (3) Peduncle colourless: proximal three-quarters yellow, distal quarter brownish yellow. (4) Proximal half yellow, distal half yellowish brown. (5) Proximal two-thirds yellow, distal third yellowish brown. (6) Proximal third yellow, distal two-thirds yellowish brown, or unicolorous yellowish brown. (7) Yellowish brown.

T. fuscipennis Haliday.

Female (Plate VII, fig. 6).

Length about 1·1 mm. General colour light greyish brown, or orange brown to dark brown.

(*a*) Ocellar crescents orange or red.

(*b*) Setae at lateral posterior margin of prothorax of moderate length (Text-fig. 9 B).

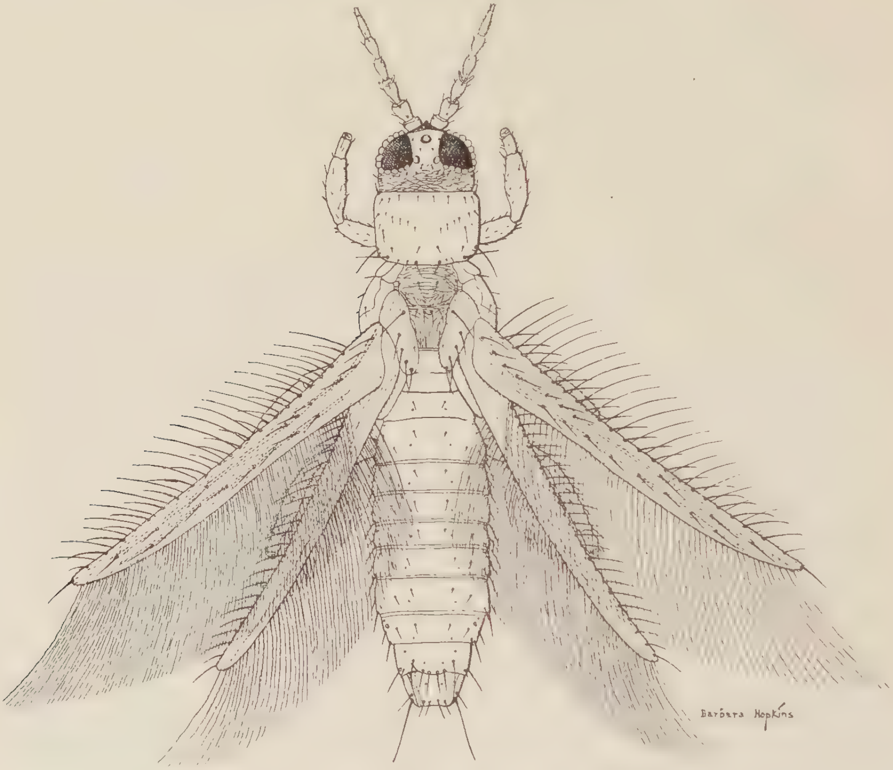
(*c*) Along extreme lateral margins of second abdominal tergite, four setae (with an anteriorly placed seta-pit) (Text-fig. 1 A).

(*d*) Posterior margin of eighth abdominal tergite straight: dorsal portion of the margin devoid of a fringe of setae: laterally only, a fringe of short fine seta arranged at irregular intervals to each side (Text-fig. 8 B).

(*e*) Ninth abdominal tergite with an anterior (placed dorsally or laterally) and a posterior pair of seta-pits (Text-fig. 8 B).

(*f*) First abdominal sternite with two minute but conspicuous setae placed upon the anterior margin, one on either side of the middle line (Text-fig. 1 A).

(*g*) Sterites and epipleurites of the second to seventh abdominal segments, inclusive, devoid of intermarginal setae (Text-fig. 1 A).



Text-fig. 6. *T. physapus* L. Male. Dorsal view. × 75.

Male (Plate VI, fig. 2).

Length about 0·9 mm. General colour greyish yellow to yellowish brown. Characters (*a*), (*b*), (*c*), (*e*), (*f*) and (*g*) as in female. Ninth abdominal tergite dorsally with one pair of anterior setae, and four posterior setae arranged in a transverse row (Text-fig. 3 A).

Translucent depressions present upon the third to seventh abdominal sternites inclusive: of moderate size and somewhat elongate transversely (Text-fig. 2 B). Colour of antennal joints: (1) Greyish yellow. (2) Proximal half light yellowish brown, distal half yellow. (3) Peduncle grey with a narrow colourless transverse band at its apex: unicolorous light brownish yellow, sometimes slightly shaded near the base (distal to the peduncle) and in the distal half. (4) Unicolorous brownish yellow, sometimes yellow in the proximal third, brownish yellow in the distal two-thirds. (5) Unicolorous yellowish brown, often yellow at the extreme base. (7) Dark yellowish brown.

T. major Uzel.

Female (Plate VII, fig. 7).

Length about 1·1 mm. General colour orange-brown to brown.

(*a*) Ocellar crescents orange or red.

(*b*) Setae at lateral posterior margin of prothorax of moderate length (cf. Text-fig. 9 B).

(*c*) Along extreme lateral margins of second abdominal tergite, three setae (with an anteriorly placed seta-pit) (cf. Text-fig. 1 B).

(*d*) Posterior margin of eighth abdominal tergite straight: dorsal portion of the margin devoid of a fringe of setae: laterally only, a fringe of short fine setae arranged at irregular intervals to each side (cf. Text-fig. 8 B).

(*e*) Ninth abdominal tergite with an anterior (placed dorsally or laterally) and a posterior pair of seta-pits (cf. Text-fig. 8 B).

(*f*) First abdominal sternite devoid of setae (cf. Text-fig. 2 C).

(*g*) Sternites and epipleurites of the second to seventh abdominal segments, inclusive, devoid of intermarginal setae (cf. Text-fig. 1 A).

Male (Plate VI, fig. 3).

Length about 0·8 mm. General colour yellow to greyish yellow. Characters (*a*), (*b*), (*c*), (*e*), (*f*) and (*g*) as in female. Ninth abdominal tergite dorsally and anteriorly with a lateral pair and a median pair of setae arranged in a transverse row: posterior margin with a single pair of setae, lateral to the median line (Text-fig. 3 B). Translucent depressions present upon the third to seventh abdominal sternites inclusive: of moderate size and somewhat elongate transversely (Text-fig. 2 C). Colour of antennal joints: (1) Colourless. (2) Colourless. (3) Peduncle grey with a narrow colourless transverse band at its apex. Light yellow, sometimes shaded at the apex. (4) Proximal two-thirds light yellow, distal third brownish yellow. (5) Proximal half yellow, distal half yellowish brown. (6) Proximal third yellow, distal two-thirds yellowish brown. (7) Yellowish brown.

T. physapus L.

Female (Text-figs. 4 and 5).

Length about 1·3 mm. General colour brown to dark brown.

(*a*) Ocellar crescents dark reddish brown.

(*b*) Setae at lateral posterior margin of prothorax long.

(*c*) Along extreme lateral margins of second abdominal tergite, three setae (with an anteriorly placed seta-pit) (Text-fig. 1 B).

(*d*) Posterior margin of eighth abdominal tergite straight; beset with a complete fringe of rather coarse setae, arranged far apart at regular intervals.

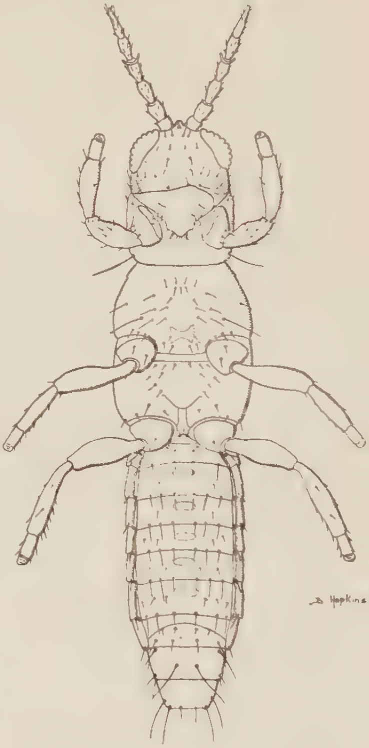
(*e*) Ninth abdominal tergite with an anterior and a posterior pair of seta-pits (cf. Text-figs. 8 B and C).

(*f*) First abdominal sternite with three minute but conspicuous setae, placed anteriorly between the metathoracic coxae, and arranged as at the corners of a triangle (Text-fig. 5).

(*g*) Epipleurites of the second abdominal segment with a short seta placed anteriorly near the margins adjoining the tergite on each side (Text-fig. 1 B).

(*h*) Sternite of the second abdominal segment devoid of intermarginal setae (cf. Text-fig. 1 A).

(*i*) Sternites of the third to seventh abdominal segments (inclusive) each with an irregular row of from about 10 to about 20 intermarginal setae (Text-fig. 1 B).



Text-fig. 7. *T. physapus* L. Male. Ventral view. ×75.

Male (Text-figs. 6 and 7).

Length about 0·9 mm. General colour light brown. Characters (*a*), (*b*), (*c*), (*e*), (*f*), (*g*) and (*h*) as in female. (*i*) Sternites of third to eighth abdominal segments (inclusive) each with 2–6 intermarginal setae (Text-fig. 7). Translucent depressions present upon third to seventh abdominal sternites inclusive: of large size, not greatly elongated transversely (Text-fig. 7). Colour of antennal joints: (1) Light brown. (2) Light brown, or the distal third to half yellow. (3) Peduncle and joint yellow. (4) Yellow, slightly shaded in distal quarter. (5) Proximal half yellow, distal half brownish yellow, or unicolorous brownish yellow. (6) Proximal half yellow, distal half yellowish brown; sometimes unicolorous yellowish brown. (7) Yellowish brown.

T. validus Uzel.

Female.

Length about 1·3 mm. General colour light to dark brown.

(*a*) Ocellar crescents dark reddish brown.

(*b*) Setae at lateral posterior margin of prothorax long.

(*c*) Along extreme lateral margins of second abdominal tergite, four setae (with an anteriorly placed seta-pit).

(*d*) Posterior margin of eighth abdominal tergite straight; beset with a complete fringe of setae, unequal in length and arranged at irregular intervals.

(*e*) Ninth abdominal tergite with an anterior and a posterior pair of seta-pits.

(*f*) First abdominal sternite with three minute but conspicuous setae, placed anteriorly between the metathoracic coxae, and arranged as at the corners of a triangle (one or other of these setae occasionally absent).

(*g*) Sternites and epipleurites of the second to seventh abdominal segments, inclusive, devoid of intermarginal setae.

Male.

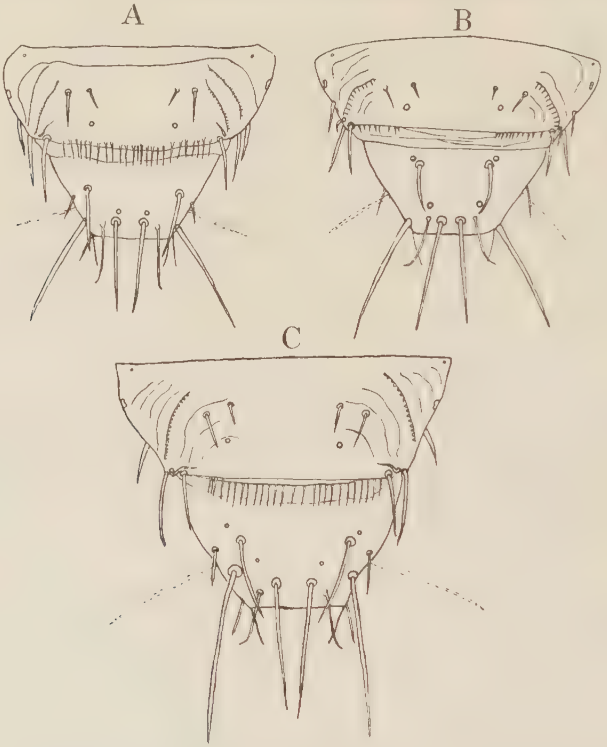
Length about 0·9 mm. General colour light brown. Characters (*a*), (*b*), (*c*), (*e*), (*f*) and (*g*) as in female. Translucent depressions present upon third to seventh abdominal sternites inclusive: very small, round. Colour of antennal joints: (1) Light brown. (2) Light brown or distal third to half yellow. (3) Peduncle and joint yellow. (4) Yellow, slightly shaded in distal quarter. (5) Proximal half yellow, distal half brownish yellow: or unicolorous brownish yellow. (6) Unicolorous brown or proximal half yellow, distal half yellowish brown.

Amongst the numerous specimens of the above-named species which have been examined, an occasional individual has shown some slight variation in one or other of the characters set out:

T. fuscipennis Hal. ♀. One specimen with an asymmetrically placed intermarginal seta upon the third abdominal sternite. In many specimens a seta-pit is present upon some or all of the abdominal epipleurites. Rarely the minute seta of one side only is missing upon the front margin of the first abdominal sternite.

T. tabaci Lind. ♀. One specimen with a pair of intermarginal setae upon the fifth abdominal sternite.

Though apparently quite constant for the above six species, the number of setae placed along the lateral margins of the second abdominal tergite is slightly variable in *T. minutissimus* L., in the female of which there is not infrequently a small additional seta, making five in all, though never less than four have been present in any specimen so far examined. Certain species are only separable from some others with considerable difficulty, and upon very few characters.



Text-fig. 8. Eighth and ninth abdominal tergites. × 175. A, *T. tabaci* L. Female. B, *T. fuscipennis* H. Female. C, *T. flavus* S. Female.

T. difficilis Pr. much resembles *T. major* Uzel: in the male, however, the setae upon the ninth abdominal tergite are arranged as in the male of *T. fuscipennis* Hal.; the female of *T. difficilis* Pr. resembles very closely the female of *T. major* Uzel and the only constant character which apparently separates it from that species is the shortness of the setae at the posterior lateral margins of the prothorax. These are no longer than

Table I.

Diagnostic characters for ten common species of the genus Thrips.

	physapus L.	*minutissimus* L.	*praetermissus* Pr.	*angusticeps* Uz.	*validus* Uz.	*fuscipennis* Hal.	*viminalis* Uz.	*major* Uz.	*flavus* Schr.	*tabaci* Lind.
For both sexes.										
Head										
Ocellar crescents red	×	×	×	×	×	×	×	×	×	.
Ocellar crescents grey	.	.	.	.	.	.	.	.	.	×
Prothorax										
Setae at post. lateral margin long	×	.	.	.	×	.	.	.	×	.
Setae at post. lateral margin of moderate length	.	×	.	×	.	×	×	×	.	.
Setae at post. lateral margin short	.	.	×	.	.	.	.	.	.	×
Abdomen										
No. of setae along side margins of second tergite	3	4–5	3	3	4	4	3	3	4	3
Two pairs of seta-pits on ninth tergite	×	×	×	×	×	×	×	×	×	.
One pair of seta-pits on ninth tergite		.	.	.	.	.	.	.	.	×
No. of setae at ant. margin of first sternite	3	2	3	2	3	2	3–4	0	0	0
Sternites 2–7 without intermarginal setae	.	.	.	.	×	×	×	×	×	×
Sternites 2–7 with intermarginal setae	.	.	.	×	.	.	.	.	.	.
Sternites 3–7 with intermarginal setae	×	×	×*	.	.	.	.	.	.	.
Epipleurites of second segment with one seta	×	.	.	.	.	.	.	.	.	.
Epipleurites of second segment devoid of setae	.	×	×	×	×	×	×	×	×	×
Epipleurites of third to seventh segments with intermarginal setae	.	×	×	.	.	.	.	.	.	.
Epipleurites of third to seventh segments without intermarginal setae	×	.	.	×	×	×	×	×	×	×
For females only.										
Eighth abdominal tergite										
Posterior margin straight	×	×	×	×	×	×	.	×	×	×
Posterior margin bluntly serrate	.	.	.	.	.	.	×	.	.	.
Fringe of setae across post. margin complete	×	×	×	×	×	.	.	.	×	×
Fringe of setae across post. margin interrupted dorsally	.	.	.	.	.	×	×	×	.	.
Setae across post. margin fine and close together	.	×	×	×	.	.	.	.	×	×
Setae across post. margin coarse and far apart	×	.	.	.	.	.	×	.	.	.
Setae across post. margin placed at irregular intervals	.	.	.	.	×	×	.	×	.	.
For males only.										
Ninth abdominal tergite										
With two anterior and four post. setae	×	×	×	×	×	×	×	.	×	×
With four anterior and two post. setae	.	.	.	.	.	.	.	×	.	.
Sternal depressions										
Present on abd. segments 3–7 inclusive	×	×	×	×	×	×	.	×	×	.
Present on abd. segments 3–6 inclusive only	.	.	.	.	.	.	×†	.	.	.
Present on abd. segments 3–5 inclusive only	.	.	.	.	.	.	.	.		×
Large and transversely elongate	.	.	.	.	.	.	.	.	×	.
Of moderate size and transversely elongate	.	×	×	×	.	×	.	×	.	.
Of moderate size and oval	.	.	.	.	.	.	×	.	.	.
Very small and round	.	.	.	.	×	.	.	.	.	.
Transversely very long and very narrow	.	.	.	.	.	.	.	.	.	×

* A single intermarginal seta is sometimes present upon the second abdominal sternite.

† A vestigial depression is sometimes present also upon the seventh abdominal sternite.

in *T. tabaci* Lind. The antennae are much shorter and the number of costal setae less than in *T. major* Uzel, but until a larger series is available, it would be unsafe to separate the two upon the latter characters which are known to be variable in other species. *T. sambuci* Uzel only differs, in the female sex, from *T. fuscipennis* Hal. by the entire absence of a fringe of setae upon the hind margin of the eighth abdominal tergite, and in the greater breadth of the third, fourth, and fifth antennal joints. Until males are available for examination, these characters are not considered sufficient to separate the two species. In Table I the most constant specific characters are set out for ten species which are likely to be met with in Great Britain.

For reference to previous descriptions, the numbers of setae (macrotrichia) upon the costal margin and distal portion (the number in the proximal portion seldom varying from the formula 4 + 3) of the fore-vein are given for females of various macropterous species, with the approximate length of the setae at the posterior lateral margins of the prothorax.

Table II.

Number of setae upon the fore-wing veins, and length of prothoracic setae in females of ten species of the genus Thrips.

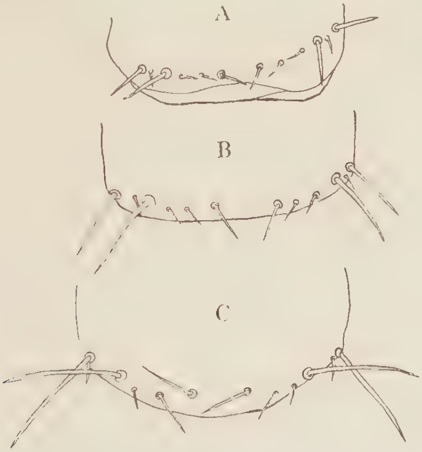
	Macrotrichia upon the fore-wing				
	Costal margin		Distal portion of fore-vein		Prothoracic setae Approx. length
Species	Approx. range	Usually	Approx. range	Usually	
T. physapus L.	26–32	28	2–4	3	80μ
T. minutissimus L.	24–31	29	7–14	11	70μ
T. praetermissus Pr.	21–29	?	?	3	50μ
T. validus Uz.	23–30	27	2–4	3	80μ
T. fuscipennis Hal.	21–29	26	2–5	3	60μ
T. viminalis Uz.	?	25	2–4	3	60μ
T. major Uz.	24–33	30	2–4	3	60μ
T. difficilis Pr.	19–23	?	—	2	40μ
T. flavus Schr.	24–30	28	2–5	3	90μ
T. tabaci Lind.	25–30	28	2–6	4	40μ

Synonymy.

T. praetermissus Pr.	*Die Thysanopteren Europas*, Vienna 1926–8.
T. montivagus Pr.	,, ,, ,, ,, ,,
T. pillichi Pr.	,, ,, ,, ,, ,,
T. ? kerschneri Pr.	,, ,, ,, ,, ,,

X. NOTES UPON THE BIOLOGY OF COMMON SPECIES OF *THRIPS*.

The life histories of species of the genus *Thrips* conform to those of the Terebrantia in general. Their comparatively large rounded or kidney-shaped eggs are deposited within the tissues of the foliage or of the petals of flowers upon which the larvae will subsequently feed. The larvae are yellow, in a few species red, in colour, with deeply pigmented compound eyes made up of a few large facets. Ocelli are not present. The forwardly directed antennae are composed of six joints. As in the adult, the mouth-parts are asymmetrical, and adapted for rasping the surface of the plant tissues as well as for extracting the cell-sap. There are two larval stages, separated by a single ecdysis. At least in the second stage, the larvae are capable of jumping to considerable distances, as well as of running with rapidity, but there is a tendency to a gregarious habit of feeding within a localised area. When full-fed, the larvae of some species jump or fall to the ground where they hide in rubbish or bury themselves in the soil: here the insect changes successively to the prepupal, with short wing-cases, and the pupal stage, in which the wing-cases are fully developed, and the antennae thrown back over the head. In other species, these stages are passed through upon the food plant, and such differences in habit affect the nature of control measures to be adopted against different injurious species, this making accurate identification of great importance.



Text-fig. 9. Setae upon the posterior margin of the prothorax. × 175. A, *T. tabaci* L. Female. B, *T. fuscipennis* H. Female. C, *T. flavus* S. Female.

Within the genus *Thrips*, the imagines most frequently met with have two pairs of narrow, pointed wings, capable of propelling the insects to considerable distances in flight, though the habit of jumping is largely used as a means of locomotion. Less commonly adults of a single species may appear in three different forms, the normal with fully developed wings, termed macropterous, a second with the wings much reduced in length, known as brachypterous, and a third entirely apterous. The last-named condition is correlated with an absence of ocelli upon the head, and a modification in the structure of the meso- and meta-thorax, known together as the pterothorax.

Imagines both of separate and of the same species are distinctly gregarious in habit, and will often collect in great numbers within a single flower. Caution must therefore be exercised in placing males with their correct females, especially as females of one species appear in some cases to avoid those of another. It is, for instance, rare to find females of *T. fuscipennis* Hal. in company with females of *T. tabaci* Lind., though males of the former species congregate readily with the latter parthenogenetic species.

Whereas many species, through the rarity of their males, show a marked tendency to reproduce parthenogenetically, an exaggerated impression of this capacity may arise through the habit of the insect during the winter months.

In autumn the very numerous males of some species disappear; in the following spring females only are found ovipositing, and their offspring, consisting of both sexes, do not become mature until summer. As, however, immature females may occur in spring, it is probable that a few overwinter without previous fertilisation, in which case it appears that the unfertilised eggs subsequently laid by them develop, and give rise to offspring of the male sex only.

T. tabaci Lindeman (Plate VI, fig. 1; Plate VII, fig. 5).

From the severity of its attacks upon the foliage of the onion plant in both temperate and tropical regions, this insect is commonly known as the Onion Thrips. Its depredations extend no less to tobacco, cotton, and potato.

Greenhouse plants are especially addicted to injury, for the Thrips breeds freely upon the foliage of the cucumber and cineraria, and within the flowers of carnation, arum, cyclamen; and adults frequently collect together in the flowers of chrysanthemum during the winter months, and by their feeding cause damage especially to blooms of the bronze varieties. During the winter eggs may be laid in the petals of rose blooms grown under glass, but to this crop the insect is of no economic importance.

Out-of-doors, in England, the *Thrips* does not become common upon cultivated or wild plants until July, and larvae have been taken from flowers of yarrow at the beginning of November. In autumn the adults congregate with other species in the flowers of rose and of various other cultivated plants, and sometimes in those of the dandelion.

Reproduction in this species is almost entirely parthenogenetic: the only authentic record of the occurrence of males comes from Scotland, where Dr G. Morison collected them from *Cochlearia officinalis* at Banchory, Kincardine, and from *Senecio jacobaea* at Cullen, Banffshire, in August. The female imago appears to hibernate in turf, as severe attacks have occurred already at the end of December in cucumber houses, in which this material had been incorporated in the borders. Early outbreaks of this nature die down at the completion of one generation; later the periodicity in occurrence of the larvae is evidently due to their dropping to the ground, where the pupal stages are passed within pieces of straw.

Breeding seldom occurs extensively upon the tomato plant, but the adult insects live in the soil during winter and spring, and feed sporadically upon any young foliage which is available. It is not improbable that common forms of mosaic disease may be disseminated through this habit.

Miss E. I. MacGill (1927) gives the duration of the life cycle upon the cotton plant grown under glass at a mean temperature of 66·2° F. as follows:

Egg 8 days; larval stages 10-14 days; prepupa 1-2 days; pupa 4–7 days: total about 4 weeks. Seven parthenogenetic generations are recorded by her during the summer. The insect has been observed to breed throughout the year upon the flowers of arum, grown under glass, without the appearance of the male sex upon any occasion.

T. fuscipennis Haliday (Plate VI, fig. 2; Plate VII, fig. 6).

This species is locally a serious pest of roses and of the cucumber plant in greenhouses. In the case of roses, the eggs are laid in the outer petals of the flowers, as soon as the calyx sepals begin to separate, at which time the parent females feed at the apex of the flower bud, causing subsequent malformation of the majority of petals when the bloom opens. Eggs are also laid extensively in the foliage of weak shoots, known to the grower as "water-shoots." The corolla of the cucumber flower is chosen for oviposition, and the flower being short-lived, the larvae, on hatching, move to the foliage, which sustains considerable injury, though the flowers show no signs of any damage. The larvae do not leave the plant prior to pupation, for which a favourite place is a hollow stem-end left after the cutting of the fruit. In cucumber houses this species has not been found in company with *T. tabaci* Lind., and the latter insect only occurs at all frequently in rose flowers when the former is absent during the winter months. In autumn, adults of both sexes may occur in vast numbers upon various flowers out-of-doors, as well as upon those of roses under glass, but all disappear in November, and it is suspected that the females hibernate, irrespective of temperature conditions, either in crevices in the structure of the greenhouse, or deeply buried in the ground. In glasshouses, oviposition begins at the end of February, the first generation being completed by the beginning of April. Out-of-doors, females start laying eggs probably not before the middle of May, shoots of apple and hawthorn being chosen for the purpose. In summer both sexes are extremely abundant in flowers of rose, convolvulus, larkspur, lupin, and many wild plants, especially buttercup. Under greenhouse conditions, the period occupied in the various stages of the life history are almost exactly similar to those recorded above for *T. tabaci* Lind.

T. major Uzel (Plate VI, fig. 3; Plate VII, fig. 7).

Females of *T. major*, which superficially so closely resemble those of *T. fuscipennis* Hal., have been collected in large numbers from the flowers of *Viburnum* in the middle of May, and sparingly from those of *Choisya* and red hawthorn, but a female was taken from *Berberis* flower at the end of April. Larvae were present in the *Viburnum* flowers at the beginning of June, but as no pupal stages were found subsequently and males made their appearance toward the middle of that month, it is assumed that pupation occurs in the ground. Both sexes are common during the summer months, especially in clover flowers, and are found in company with *T. fuscipennis* Hal., though only the more active males have been collected from roses grown under glass, and these late in summer and in very small numbers. A single female was taken, in company with numerous *T. flavus* Schrank, from a flower of *Magnolia* at the end of September. This and the following species do not appear to be of any economic importance.

T. flavus Schrank (Plate VI, fig. 4; Plate VII, fig. 8).

A larger insect than *T. tabaci* Lind. which it somewhat resembles superficially. Males were collected from various white flowers, a female from *Buddleia*, during late summer, and both sexes from rose blooms in autumn. Young stages have not been met with, but Dr Guy Morison kindly sent larvae collected from gorse in Berkshire (September).

T. physapus Lindeman (Text-figs. 4–7) and *T. validus* Uzel.

Common in flowers of dandelion from June to September. Both often occur together in the same flower. Whereas males of both species outnumbered their respective females in September, males of *T. validus* preponderated over females in May and June, the reverse at this time being the case with *T. physapus*. The red larvae of *T. validus* were found in the flowers in May and again in July, but neither pupae of this species or of *T. physapus* have been observed. It is probable that Priesner's description of the larvae of *T. physapus* refers to that of *T. validus*. In company with these species, numerous males and few females of *T. praetermissus* Pr. have been collected in September, and many females from white yarrow flower. Larvae much resembling those of *T. angusticeps* Uzel, but probably of this species, occurred in a dandelion flower in June.

XI. SOME REMARKS UPON THE PRESENT CLASSIFICATION OF THE THYSANOPTERA.

Although so few species, and those of only a single genus, have been considered here, the material presented may suffice to indicate that characters of a very variable nature have been chosen in the past as a basis for the classification of the Thysanoptera; much the same system has been employed for the separation of genera and even families as has been exploited for that of the species. This does not signify that a character which is too variable to be relied upon for the separation of species may not become of value when applied in a broader sense to genera and families. As an example, the distinctive differences in the shape of the head in such genera as *Dendrothrips*, *Chirothrips* and *Anaphothrips* cannot be disregarded, though smaller differences amongst the species may be inconstant as a specific character within each genus.

Amongst the Terebrantia, there appears to be a curious absence of correlation in structural differences. A group of species with a single well-marked character will seldom exhibit any further peculiarities which are not common to the species of other groups sometimes far removed from it. The consequences of this lack of structural correlation lead to the finding of a repetition of the same specific differences amongst the species of various genera. Parallel lines of evolution are therefore indicated amongst the species of such genera. Another noteworthy feature of the Terebrantia is the frequency of a very close superficial resemblance between the adult females of different species, their respective males being quite unlike. It would appear that evolutionary forces have acted upon the male to a far greater extent than upon the female sex, unless those forces have led to chance convergence amongst the females only. Repetition and convergence of structure undoubtedly enhance the difficulties of achieving a sound classification of the Thysanoptera, especially in view of the comparative rarity of males. Unfortunately, differences in the number of joints of which the antennae are composed have been relied upon as a distinctive generic character, largely to the exclusion of structures which present far more constant features.

In the admittedly small number of instances in which males are available for examination, it would appear that the genitalia are useful for the separation of groups not higher than genera. A study of the male genital structures would doubtless lead to a profound modification of the existing classification.

As reconstituted by Priesner (1926), the genus *Taeniothrips* now includes at least two groups of species in which these structures are entirely different from one another. For this reason, the very common species *Taeniothrips atratus* Hal. and *T. vulgatissimus* Hal. should not be included in the same genus with *T. picipes* Zett., the type species of this genus. The two former species, despite the fact that the antennal style (*i.e.* the small terminal joint or joints) is divided, show close affinities with *Thrips physapus* L. and other species of the genus *Thrips* related to its type species.

The number and position of strong setae upon the prothorax provide characters of greater generic value than the antennal structure, with the exception of the disposition and nature of the sensillae or sensory areas. In cases where the latter structures are similar, differences in the antennal style should not be regarded as of any significance for purposes of classification.

At least amongst the Thysanoptera, the ever-growing confusion is due to meagreness of description for the generic character. However true the contention that the genus is a group of somewhat similar species, assembled together for the sake of convenience rather than philogenetic affinity, there is no justification for a system of classification based upon structures of the insect which happen conveniently to strike the eye, with no primary inquiry into the possibility of such structures being highly variable in the species. The scientific examination of structure has of late given way to short-cut methods of mechanical measurement, relating to portions of the external anatomy for which no attempt at proof of constancy in the species has been undertaken. The time is now at hand for a complete cessation of description relating to new genera and species. The confusion that has arisen can be unravelled only by reference to the *type species* of such genera as have already been raised, for these are the only definitely *fixed* points which are available to the systematist.

In not a few cases the actual type specimen representing the genus may not be available, or is in a state of preservation which defies examination, but in the majority of cases the type of the genus is represented by a species not only well recognised, but of which fresh material can without difficulty be obtained. There is now occasion for the systematist to determine from series of specimens what characters are liable to most and what characters to least variation in the type species of each genus, and from the latter category and from a similar examination of related species to choose constant characters which are serviceable for the separation of species within the genus. Only after such an undertaking is complete will it be possible to raise genera and higher groups for the final constitution of a justifiable classification, of practical value for the systematist and satisfactory to the needs of the biologist.

XII. SUMMARY.

1. The economic importance of the Thysanoptera as an order of insects and the necessity for a revision in the classification of the order are pointed out.

2. The past confusion between some common and destructive species of the genus *Thrips* is unravelled and the reasons for this confusion are set out.

3. The range of variation, within the species, of characters which have in the past been used for the separation of species in the genus *Thrips* is illustrated, this relating especially to measurements of the antennal joints and of setae upon the body. New structural characters of true specific value, especially relating to the chaetotaxy of the abdomen, are illustrated by examples.

4. Variation in colour and the shape of the head are referred to.

5. Characters of a constant nature for the species and of value in determination are set out, together with some account of the abdominal structure.

6. Quick and reliable methods for the mounting of specimens for microscopic examination are given.

7. Specific characters, which enable them to be distinguished from one another, and from other species, are given for some common British species of the genus *Thrips*.

8. A general account of the biology and a more detailed account of that relating to some common species is given.

9. Lines upon which a sounder classification of the Thysanoptera than at present exists are outlined.

XIII. ACKNOWLEDGMENTS.

Especially are thanks due to Mr F. Laing, of the British Museum of Natural History, for invaluable help and advice, and for provision of facilities for the examination of material in the National Collection; to Dr C. B. Williams, of the Rothamsted Experimental Station, for similar help in greatly assisting in the preparation of the material presented. and for the loan of valuable specimens from his private collection of Thysanoptera; and to Dr Guy Morison, of Marischal College, Aberdeen, not only for the loan of important specimens, which could not be obtained elsewhere, but for a chance suggestion which initiated and made possible the whole of the systematic work here undertaken.

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EXPLANATION OF PLATES VI AND VII.

PLATE VI.

Males of the genus *Thrips.* ×50.

Fig. 1. *T. tabaci* Lind.
Fig. 2. *T. fuscipennis* Hal.
Fig. 3. *T. major* Uzel.
Fig. 4. *T. flavus* Schrank.

PLATE VII.

Females of the genus *Thrips.* ×50.

Fig. 5. *T. tabaci* Lind.
Fig. 6. *T. fuscipennis* Hal.
Fig. 7. *T. major* Uzel.
Fig. 8. *T. flavus* Schrank.

(*Received May 3rd,* 1933.)

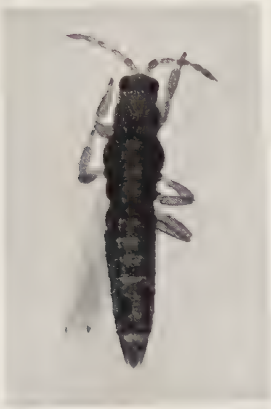

Fig. 1.

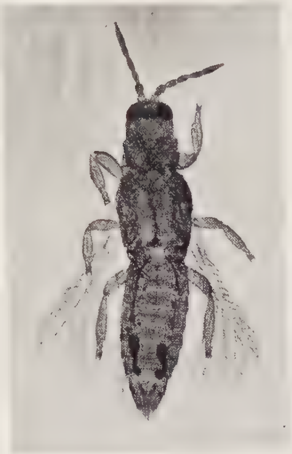

Fig. 2.

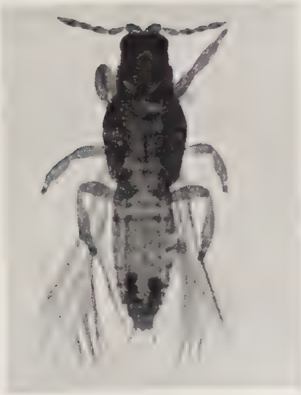

Fig. 3.

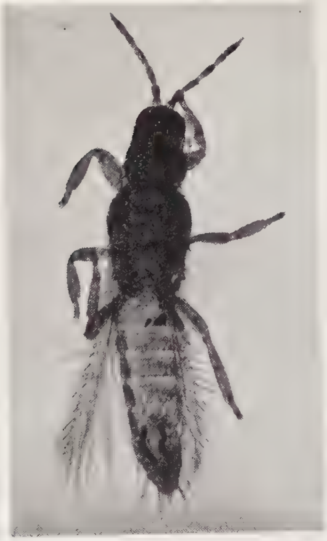

Fig. 4.

SPEYER.—Some Common Species of the Genus *Thrips* (Thysanoptera) (pp. 120–152).

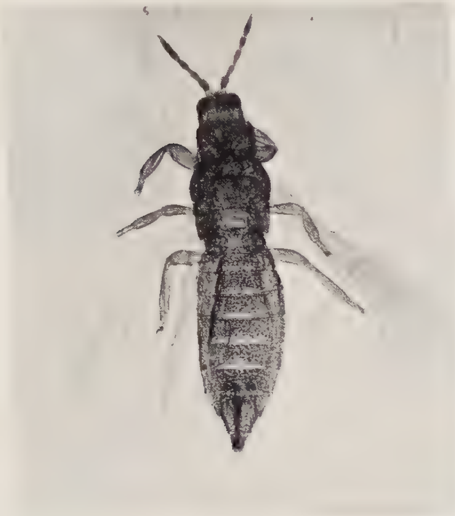

Fig. 5.

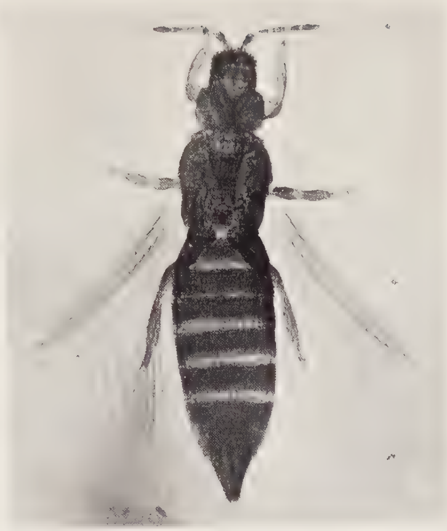

Fig. 6.

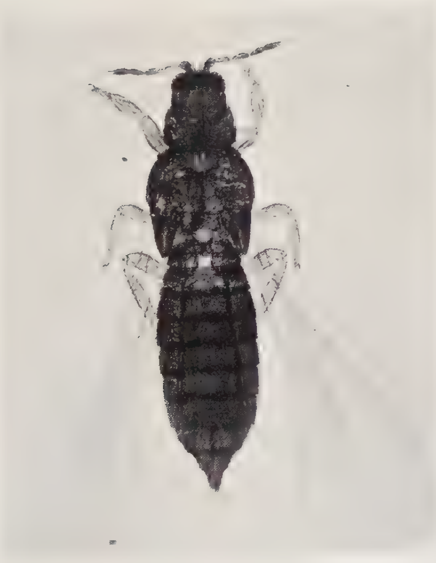

Fig. 7.

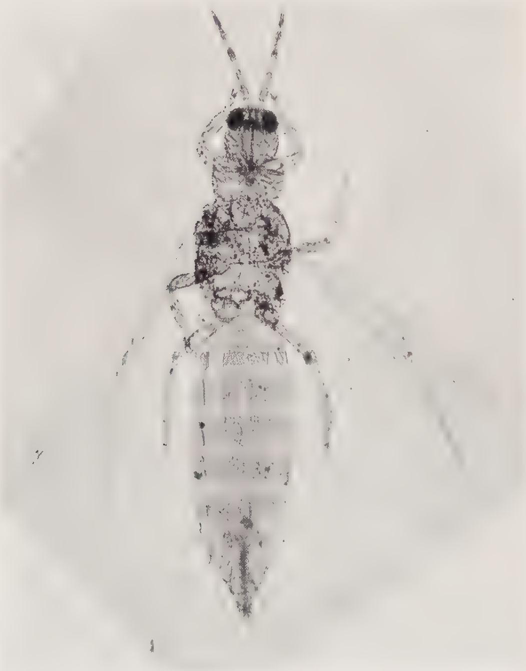

Fig. 8.

SPEYER.—Some Common Species of the Genus *Thrips* (Thysanoptera) (pp. 120–152).

ON THE BIOLOGY OF *PSYLLIODES HYOSCYAMI* LINN. (CHRYSOMELIDAE, COLEOPTERA), THE HENBANE FLEA-BEETLE, WITH DESCRIPTIONS OF THE LARVAL STAGES

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(With Plates VIII and IX and 3 Text-figures.)

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I. Introduction.

During the early summer of 1929 some specimens of Henbane (*Hyoscyamus niger* L.) were sent to this Station by Mr K. R. Reynolds from the Medicinal Herb Farm of Messrs Ransom and Son, Ltd., Hitchin. The plants were suffering from root damage, which so far as could be seen was due to millipedes and advice was given accordingly. The affected plants were kept and after a few days were noticed, in addition, to show signs of infection with numbers of Coleopterous larvae. These mined the pith, leaf stalks and leaves, and on breeding out were found to be those of *Psylliodes hyoscyami* Linn. This beetle, according to Fowler, is very rare, as a rule, and since the infestation appeared to be very heavy the farm was visited three times during 1930 and occasionally since.

The observations made together with a description of the immature stages are embodied in this note.

I should like here to thank Messrs Ransom and Mr Reynolds who gave me every facility for the work and Miss A. M. Lysaght for drawing Plate VIII, fig. 1.

II. THE ADULT INSECT (Plate VIII, fig. 1).

Like the well-known "Turnip Flea," this beetle belongs to the Halticinae, a sub-section of a family of leaf-eating beetles, the Chrysomelidae. All the members of this sub-family are distinguished by the greater or less development of enlarged and muscular hind femora, conferring on them the powers of leaping which has earned them their common name of flea-beetles. The genus, *Psylliodes*, differs from all other genera by the possession of 10-jointed instead of 11-jointed antennae. The particular species is one of the larger examples and this, together with its colour, easily distinguishes it from other Halticinae feeding on Solanaceous plants.

Fowler describes it as "oblong-ovate of a bronze green or slightly coppery colour; under side nigro-aeneous; antennae black with base reddish yellow; legs reddish yellow with posterior femur dark; length 2–4 mm." Heikertinger (1912) gives a bronzy green to bronzy brown coloration as the usual form, the var. *cupronitens* Foerst as the coppery form, and var. *coerulescens* Wse as the rare green-blue form.

Of the many specimens handled in the present case there were about equal numbers of the bronze green and the blue green variations; coppery specimens were very rare.

III. PREVIOUS RECORDS AND HOST PLANTS.

Hitherto there appear to be three recent records of *Psylliodes hyoscyami* attacking medicinal crops such as henbane. Zacher (1921) records the beetle as attacking henbane in Bohemia in company with *Epitrix atropae* Foudr. and *Psylliodes affinis*, the two last named also attacking *Atropa Belladonna*. In the Crimea, Perfentjev (1921) states that though it is occasionally found on belladonna, *Psylliodes hyoscyami* more frequently attacks henbane. Van Emden (1924), in a survey of insects attacking medicinal plants in Saxony, also gives belladonna as a host plant in addition to henbane. Heikertinger (1925) considers this *Psylliodes* to be confined to *Hyoscyamus niger* in the neighbourhood of Vienna, but thinks that farther south it might be found on other species.

In the present instance, though a strip of belladonna adjoined the heavily infested henbane, few beetles were found on it, nor did they move over when the henbane was harvested in June. In the laboratory, however, beetles would eat belladonna leaves when given no other choice; *Solanum dulcamara, S. tuberosum* and *Nicotiana* sp. were refused.

IV. LIFE CYCLE AND HABITS.

During the winter the beetles, unlike *Psylliodes chrysocephala*, did not appear to be active but were found hibernating in the long grass bordering the field. By the end of April, though the weather was still very cold, large numbers were found collected on the roots and stems of young *Hyoscyamus* plants which had been partially buried when the land was ploughed. These, when taken to the laboratory, began oviposition during the first week of May. The eggs (Plate VIII, fig. 3 E) appear to be laid in the soil, as a search in the field showed none on the leaves or stem. That the soil is the normal place of oviposition is indicated by laboratory experiments with potted henbane plants. At the same time the surface of the *Hyoscyamus* is very hairy and covered with a sticky secretion. It is difficult to believe that the young larvae can travel over such a surface which so evidently entraps small Diptera. In any case the cast skins of first instar larvae have been found in the mines in upper regions of the plant. The eggs hatch in about a fortnight (June 14th–28th).

The whole of the larval life is passed within the plant (Plate IX, figs. 4, 5), the leaf-stalks of which are mined almost to the leaf tip, and in cases of overcrowding mines in the blade of the leaf are formed. The central pith of the main stem is also invaded and some larvae were found in the tap root. In bad infestations the stem is completely hollowed out.

Egg laying continues during May and June. By the second week of June, 1930, the oldest larvae were full grown and left the plant to enter the soil where the pupal and prepupal stages are passed (Plate VIII, fig. 3 R, P). The prepupa constructs the usual earthen cell, lining the inside surface with a cementing secretion. The majority of the pupae lie between 2 and 3 in. deep. The first were seen on June 21st and the first of the new generation emerged at the end of July; the maximum numbers a few days afterwards.

There appears to be only one generation a year and a number of the new generation were dissected at various times during the summer and early autumn, but no ripe ova were found nor were any eggs laid in captivity. It is probable that oviposition does not normally occur till the following spring. In this respect, therefore, *Psylliodes hyoscyami* differs from *P. chrysocephala* the larvae of which mine the stems of Brassicae. That species oviposits during the autumn and winter so that the winter is passed both in the adult and immature stages.

V. Associated insects.

In plants which had been attacked by the beetle larvae for some time and where rotting of the pith had begun, numbers of Cyclorrhaphous dipterous larvae were found scattered throughout the length of the stem (Plate IX, fig. 6). These, on breeding out, were determined by Mr J. E. Collin as *Lonchaea flavidipennis* Zett. He expressed the opinion that they were a secondary infection and referred to a similar case reported by Mr J. E. M. Mellor of Cambridge. Mr Mellor informed me that he had obtained the larvae of the same fly from rotting stems of parsley and although *Psila rosae* could not be definitely associated as the primary parasite he believed *Lonchaea flavidipennis* to be secondary.

In addition a predaceous Coleopterous larva was present in the stems (Plate IX, fig. 7).

VI. Damage.

Although the adult beetle is capable of damaging the young plant, it is the mining habit of the larvae which is the more serious. In order to appreciate the nature of the damage the method of cultivation must be briefly described.

Henbane is a biennial. Sowing usually takes place in October, though in a bad season it may be delayed till the following spring. Two leaf crops are taken from the young plants during the first year. In the following year the plants are allowed to flower, when a further crop is taken from the flower heads and finally, during June, the whole plant is cut down at ground level. It will be seen therefore that the plant is liable to attack at several periods. The small first year plants may be attacked in the spring by the over-wintering adults and this may be followed by a larval attack as the eggs laid on them hatch. The mining of the leaves and growing point of the young plants causes a serious setback and may result in their death. In the following year attack by the over-wintered adults is not so serious, but when, as in 1930, the numbers of beetles are great, much injury is again done by the larval infestation though the plants are now full grown. Individual leaves and shoots are killed, and rotting is set up. At this stage other insects and saprophytes may follow as secondary invaders. Often the plants are killed, especially when they are also suffering from the virus diseases described by Hamilton (1932). Fortunately it appears from the experiments of this worker that the flea-beetle is not capable of transmitting the disease—at any rate in the adult stage.

VII. CONTROL.

The year during which most of these observations were made (1930) was apparently an epidemic year for many flea-beetles and the attack may have been exceptionally severe. However, where both host plant and pest are localised in distribution, eradication of the pest should be possible cheaply and easily by merely manipulating the crop.

The following are the chief points of the case under consideration. First, three areas of land are used for growing the crop, two of them adjacent—this is the infested area—the third about a mile distant. Of these three plots, one always carries first year plants, one second year plants while the third rests under a rotation crop. The two adjacent areas are in the same field, so that some henbane is always present there. Incidentally, once in three years, old and young plants are growing side by side, and this means that when the old crop is cut the beetles can migrate to the young crop. Secondly, part of the second year crop is usually left for seed, thus providing a lengthened breeding ground (for the early harvesting of the main crop in June ensures a certain measure of control as many larvae are then still within the plant). The third point is that the beetles, so far, are not present on the distant plot.

Control measures are therefore being directed towards breaking the sequence of crops on the adjacent areas, accompanied by harvesting as early and rapidly as possible and the provision made for seed production elsewhere than on the affected area.

VIII. DESCRIPTION OF THE DEVELOPMENTAL STAGES.

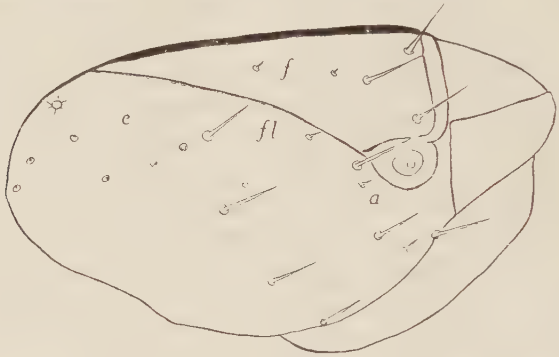
(*a*) *The egg* (Plate VIII, fig. 3 E).

The egg is oval, $\frac{2}{3}$ mm. long by $\frac{1}{3}$ mm. in width, yellowish white in colour with a deeper yellow equatorial band in the later stages. The chorion has the fine polygonal sculpturing common to flea-beetle eggs.

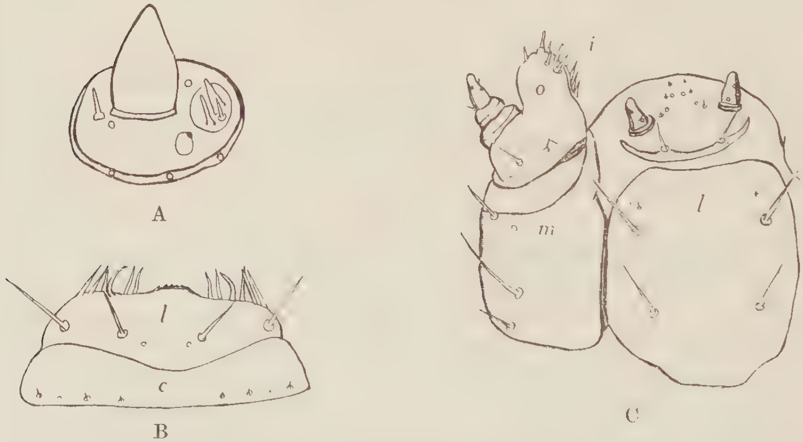
(*b*) *The larva* (Plate VIII, fig. 2).

The arrangement of the chitin plates and setae of the newly hatched larva and intermediate instars is similar to that of the full-fed larva.

The full-fed larva is of the same general appearance and elongate form as other flea-beetle larvae and the following description refers chiefly to points of taxonomic value. The body colour is white with the head dark brown, prothoracic shield, anal shield and legs light brown. Average measurements are: length 8 mm., breadth 1 mm. The arrangement of the setae on the head capsule is shown by Text-fig. 1; the antenna in Text-fig. 2 A. The strong five-toothed mandible has the usual pair of setae situated on the external face and the molar region carries four unequal sharp spines, of which the proximal is minute, projecting into the buccal cavity.

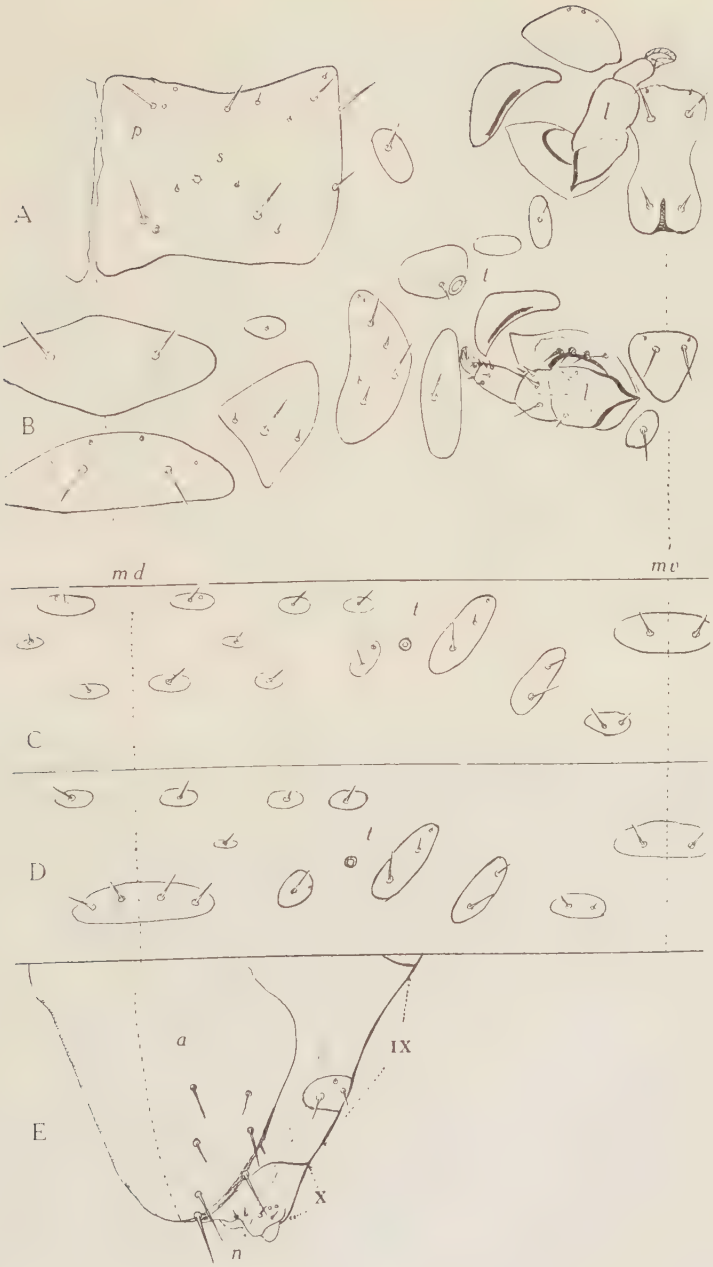


Text-fig. 1. Head capsule showing arrangement of setae. Schematic. *f*, frons; *e*, epicranium; *fl*, fronto lateral suture; *a*, antenna.



Text-fig. 2. A, left antenna and accessory sense organs. × 650. B, *l* labium and *c* clypeus. × 170. C, *m* maxilla and *l* labium. × 160. *o* outer, *i* inner lobe. × 170. Nearly full-fed larva.

Clypeus, maxilla and labium are shown in Text-figs. 2 B and C. The arrangement of the setae on the thoracic and abdominal segments is indicated by Text-fig. 3 A–E. On the prothoracic shield are two circular membranous areas (*s*) presumably sensory in function but differing from the usual form observed in flea-beetles. A pair of similar organs appears



Text-fig. 3. Arrangement of seta on the various segments. A, prothorax; B, mesothorax; C, abdominal segments 1–7; D, 8th abdominal segment; E, posterior end of larva. *p*, prothoracic shield; *s*, sense organ; *t*, spiracle; *l*, leg; *a*, anal shield; *n*, anus; IX, 9th segment; X, vestigial 10th segment; *md*, mid-dorsal; *mv*, mid-ventral line.

on the dorsal region of the epicrania of the head capsule. The metathorax is similar to the *mesothorax* except for the absence of spiracles. Abdominal segments 1–7 are similar; the 8th has the posterior dorsal plates fused, the 9th bears the anal shield, and the vestigial tenth, the anus (Text-figs. 3 C, D, E).

The pupa is of the typical flea-beetle form. It is white in colour, 4 mm. long and 2 mm. across its greater width. The chaetotaxy, which appears to be constant, is as follows. On the head three pairs of setae: on each side one below the antenna base, one at the inside margin of the eye, the third on the vertex. The prothorax bears eight pairs of setae: on each side one pair median, one antero-lateral, one median lateral, one postero-lateral and one lateral above the mesothoracic suture. Meso- and meta-thorax with four setae in a transverse line. The legs each bear two setae at the femoro-tibial bend situated externally on the tip of the femur. Abdominal segments 1–6 each with eight setae in a transverse line, the lateral seta on each side situated below the stigma on the pleura. Seventh abdominal segment shield shaped, the eight setae arranged around the posterior margin. The setae are repeated on the reduced 8th and 9th segments which are scarcely visible from above. The 9th bears the anal horns.

IX. Summary.

1. A severe attack by a flea-beetle, *Psylliodes hyoscyami* Linn. on *Hyoscyamus niger* L. grown as a commercial crop is recorded.

2. The life history and developmental stages of the beetle are described.

3. Suggestions for control are made.

4. The larvae of *Lonchaea flavidipennis* Zett. (Sapromyzidae, Diptera) has been found associated with the beetle larvae in the damaged stems.

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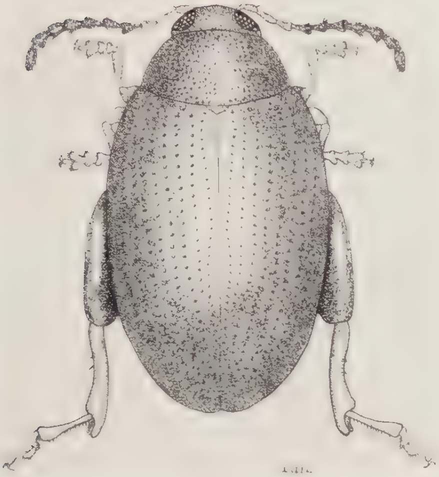


Fig. 1.

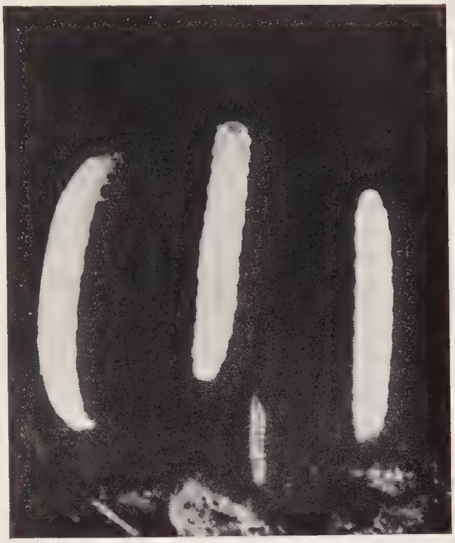


Fig. 2.

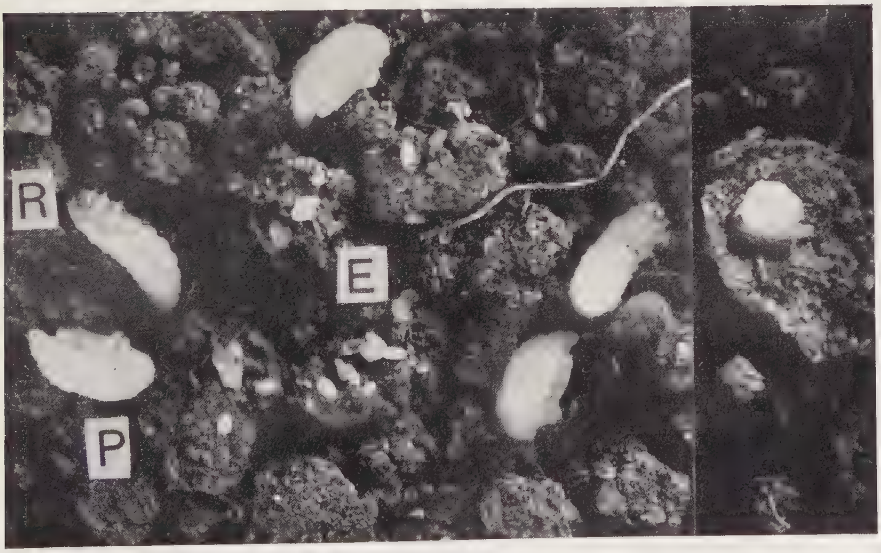


Fig. 3.

NEWTON.—On the Biology of *Psylliodes hyoscyami* Linn. (Chrysomelidae, Coleoptera), the Henbane Flea-beetle, with Descriptions of the Larval Stages (pp. 153–161).

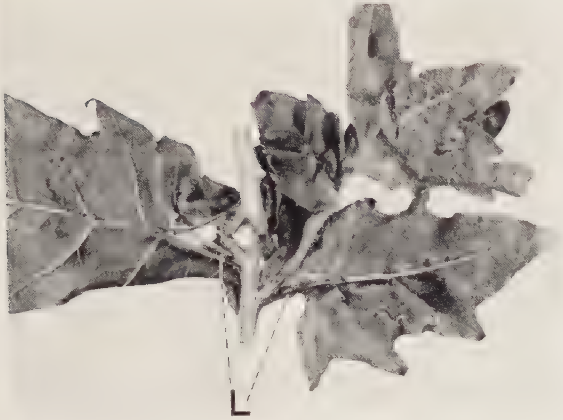


Fig. 4.

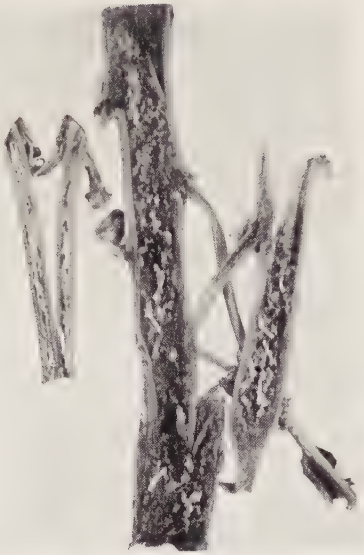
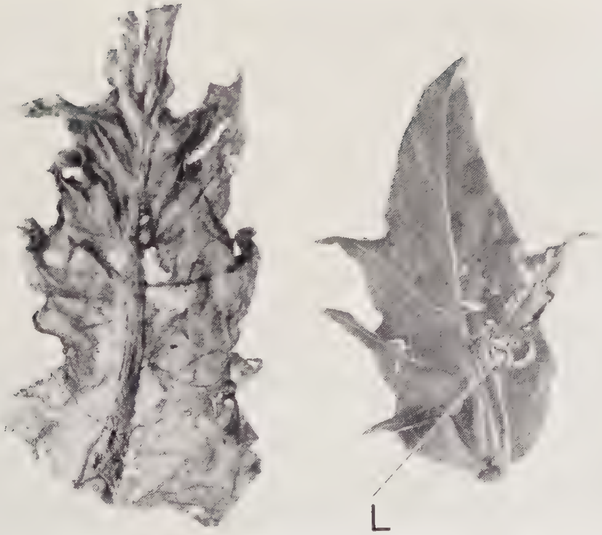


Fig. 6.



A

B

Fig. 5.

Fig. 7.

NEWTON.—On the Biology of *Psylliodes hyoscyami* Linn. (Chrysomelidae, Coleoptera), the Henbane Flea-beetle, with Descriptions of the Larval Stages (pp. 153–161).

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EXPLANATION OF PLATES VIII AND IX.

PLATE VIII.

Fig. 1. Adult beetle. × 15.

Fig. 2. Full-fed larva: side, ventral, and dorsal views. × 4.

Fig. 3. Eggs, E; prepupa, R; pupa, P. The pupa on the right still in its earthen cell. × 4.

PLATE IX.

Fig. 4. Upper region of *Hyoscyamus* stem split to show early stages of infestation in the leaf-stalks. Larvae, L.

Fig. 5. A, later stage, leaf killed. B, larva (L), mining in leaf.

Fig. 6. Split stem of an infested plant, late stage, showing larvae of *Lonchaea flavidipennis* Zett.

Fig. 7. Predaceous Coleopterous larva from mine. × 25.

(*Received March* 23*rd*, 1933.)

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

Ordinary Meeting held at 2.30 p.m. on Friday, October 20th, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Prof. W. B. Brierley.

The programme consisted of a discussion on:

"Insect and Allied Pests of Cultivated Mushrooms."

The following papers were read:

I. The Incidence of Attacks and their Relation to Growing Practice. By S. G. Jary, B.A.

II. Laboratory Investigations. By M. D. Austin, F.R.E.S.

I. THE INCIDENCE OF ATTACKS AND THEIR RELATION TO GROWING PRACTICE.

By S. G. Jary, B.A.

(S.E. Agricultural College, Wye, Kent.)

The cultivation of mushrooms is no new industry, or art (whichever one considers to be the more applicable term), but during the past few years it has developed rapidly in this country, though it has hardly sprung up in the night like the fungus itself.

Whatever other edible fungi may exist, the cultivated mushrooms are varieties of the well-known *Psalliota* (*Agaricus*) *campestris* and *P. arvensis*. Before the recent imposition of import duties on this commodity, large quantities of mushrooms were imported into this country, but with the decrease in this direction British production has enormously increased.

It may not be out of place to say a few words about the general methods of cultivation of the crop, since it will be necessary to use later on certain terms, which are more or less peculiar to the industry, in describing the processes involved and these processes have a distinct bearing upon the incidence of pest attacks.

The foundation of mushroom growing is the manure compost, consisting of stable manure, that is, horse dung and straw. This is usually not available in large enough quantities on the premises and is bought in from army stables and from town and city contractors to supplement supplies obtained locally. The raw manure is made up into large heaps, usually under some sort of covered shed and watered if too dry. Fresh wheat straw is added if it is thought that the amount of straw is too small, for the mushroom grower likes to have the manure rather "long." Fermentation soon commences in the heaps and the temperature rises to about 160° F. in the middle while the outside remains at more or less atmospheric temperature. After a few days the heap is turned; turning consists in pulling down the heaps systematically with forks, shaking any lumps to pieces and rebuilding the heaps so that the manure which was previously on the outside is thrown to the inside where, in its turn, it heats. The turning process is repeated at intervals of a few days until the compost is judged to be in the right condition, to obtain which five or six turnings may be necessary.

The final product is brown in colour, damp, but not wet enough to ooze liquid when compressed in the hand and having a distinct sweetish smell, not unpleasant or raw. There should be plenty of straw obvious in it, in pieces about 6 in. long, now brown and partially rotted. This is the compost from which the mushroom beds are made, and the beds may be either of the "flat" or "ridge" type. The compost is built into the required shape and trodden or beaten down until quite firm. Once again the temperature rises and, after reaching a peak at about 120–140° F., gradually falls again, and when it has reached about 70° F. or rather lower, the bed is ready for spawning. About 10 days often elapses between the making of the bed and spawning. Spawn consists of a sterilised compost in which the mycelium of the fungus has been grown. This is broken into small pieces about the size of a walnut, and the pieces are embedded at intervals of about a foot in the compost. They are pressed in firmly, flush with or just below the surface, and the compost is compacted around them so that a good contact is obtained. In a short time the spawn grows out and commences to run through the compost and, at any rate, the spawn should show signs of running in a week or 10 days after the insertion of the spawn. As soon as it is seen to be running, the bed is cased with soil to a depth of about 1½ in. Casing soil is usually a sandy loam subsoil, practically devoid of humus, and of such texture that it will readily bind together and bed down firmly without crumbling. This is built to the desired depth all over the beds and smoothed down to a firm surface. Periodically the casing soil is lightly watered to prevent it from drying out, but it is never made really wet. If out of doors, the beds are covered with a thick layer of straw over which is secured canvas or sacking and, if inside, the beds are either left uncovered or lightly strawed over. Cropping commences at some subsequent time depending largely upon temperature, but usually the first mushrooms appear in 6–8 weeks, and thereafter the bed may continue to crop for some months, picking taking place regularly. All sorts of buildings may be pressed into service for mushroom growing, with slight alterations in construction and, in addition to buildings constructed for the purpose, glasshouses, barns or other farm buildings, cellars, natural caves and derelict coal-mines may be used, while outdoor beds are common. In buildings, protection from direct light is arranged, and freedom from violent fluctuations of temperature or humidity is ensured by ventilation devices, insulation by layers of straw around the walls and roof, etc. The situation of the beds probably has a marked effect in determining the incidence of pest attacks and temperature is an important factor in this connection also. During the cropping period it is found that certain insect attacks are much more likely to occur where temperatures of over 60° F. occur in the buildings.

Periodic watering is kept up during the cropping period, but excessive moisture is always avoided and any measures which would involve the application of large amounts of liquid to the beds, however desirable from the point of view of the suppression of pests, are out of the question.

On a commercial scale the production of mushrooms is possible all the year round, winter crops being produced with the aid of any suitable heating apparatus in buildings, but without artificial heat beds are usually made to commence cropping in the early months of the year, and a succession arranged to carry on until the summer.

For the purposes of a rapid survey, the pests attacking mushrooms may conveniently be divided into three categories:

(1) Those which are associated with certain types of buildings and situations.
(2) Those which are associated with the manure or soil used.
(3) Those which are specific to mushrooms.

(1) *Those which are associated with certain types of buildings and situations.* The first group consists of a number of animals, usually not insects. They are mainly creatures of more or less omnivorous habit and feed upon almost any available vegetable and/or animal refuse. Perhaps the most widely distributed of these creatures are woodlice. They flourish in farm buildings beneath semi-rotten straw in damp dark situations, and find adequate hiding places in crevices of brick and woodwork, whence they come forth, mainly at night, and feed upon developing mushrooms. The characteristic damage takes the form of large holes eaten in the caps but, as a rule, only a small proportion of the crop is harmed. *Oniscus asellus* is a species commonly met with but *Porcellio scaber* and *P. laevis* may cause damage, and these two latter species have been recorded from America. Slugs and millipedes are to be met with under similar conditions and cause much the same type of damage, in fact, it is not easy to diagnose the cause simply by examination of the mushroom, for the holes are shallow and irregular in shape and frequently all these creatures may be concerned. Mice and rats are also responsible for eating mushrooms occasionally, and moreover do damage by burrowing into the beds. Apart from the direct injury they cause, all these creatures may have a further significance. They are all to some extent migratory in habit and, coming as they usually do from soil where humus is present in quantity, they may be suspected of introducing into otherwise clean beds infection by some of the serious fungus diseases which attack mushrooms. It will be readily appreciated that most of these pests are unlikely to be encountered in buildings specially erected and, in fact, they are almost entirely associated with buildings adapted for mushroom growing. In the ordinary way little more is needed than attention to general hygiene to reduce their attacks to proportions which are insignificant. Woodlice may readily be dealt with by insecticides containing pyrethrum applied either in the form of a liquid or as a dust. In spray form it may be applied in place of the ordinary routine watering given to the beds but, as already mentioned, heavy applications of liquid should be avoided. Pyrethrum has the advantage of being non-poisonous to man and domestic animals, and so may safely be used when beds are in full cropping. The control of slugs and millipedes which is ordinarily carried out on other crops by means of poison baits cannot be advised for mushrooms for obvious reasons. A further section of the fauna of mushroom beds consists of mites which are ordinarily numerous, but it is doubtful if they ought to be included in this category. We know so little about most of the acarine species concerned that it is not possible to give an account of their habits and mode of life, but it may well be that, in the case of some species at any rate, they are normal inhabitants of unhygienic buildings. Nevertheless, many are also to be found in the manure compost, and for this reason they are considered to fall in the next category. Similarly the springtails, *Collembola*, are for convenience placed in the second group.

(2) *Those which are associated with the manure or soil.* In studying the fauna of mushroom beds it is clearly important to know what is the natural fauna of the manure

used, and there is every reason to think that this will vary with the conditions under which it is kept and the treatment which it receives. Mites of various species are commonly met with in most samples of manure, some being definitely mycophagous forms and others of a predatory type, often associated with the former. The common mushroom mite, *Tyroglyphus mycophagus*, usually occurs thus together with a predatory species at present unidentified. Similarly dipterous larvae of many species are known to inhabit dung, and again some of these are probably predators. Some of the most common dipterous larvae are those of the family Phoridae, called in American literature "manure flies," and few samples of compost are examined in which these are not present in numbers, so that it would seem likely that Phorid species normally ovipost in stable manure. Flies of the same family have been frequently bred from larvae attacking mushrooms, but it remains to be seen whether these are the same as the ones found in the dung itself. If this should prove to be the case, then there is the possibility that the larvae in the manure at the time of making up the beds migrate through and subsequently attack the mushrooms. In the mushrooms themselves the larvae make tunnels in all directions and reduce the stalk and cap to a sponge-like consistency, such mushrooms being quite unmarketable. Up to 50 per cent. of some crops become affected in this way, particularly in the summer months.

The injury caused by mites takes the form of shallow irregular holes eaten in the caps and stalks and in these large colonies of mites may be found. Damage done by springtails takes the form of small lesions on the stalks, and the edges of the caps are also attacked giving them a ragged appearance, but the injury is seldom of a severe character. Both mites and springtails are often to be found inside tunnels in the caps and stalks, but these tunnels are almost certainly made by dipterous larvae and the other creatures are only casual visitors. In addition to the actual damage which they cause to the mushrooms themselves, mites and fly larvae are suspected of injuring the mycelium running in the beds. The mycelial strands are found to be cut up into short lengths, and this attack may have a serious effect on the ultimate weight of crop produced. The fauna of the manure is of particular interest because of the processes to which the manure is subjected before it becomes suitable for spawning. We have already seen that, when it arrives at the grower's premises, it is a very different substance from that finally made up into the beds, since fermentation processes have taken place and much of the material has been subjected to degrees of temperature and humidity which must exert a profound influence on most forms of life present. Thus the temperature of 160° F. which may be reached in the heap, combined with a high humidity, might be thought to produce conditions which approach those of sterilisation. Yet it must be remembered that the outside of the heap remains at more or less air temperature, and some forms of life may be able to migrate toward the outside. It is true that at each turning the outer portions are thrown into the middle of the newly made heap, but the temperature can only rise so slowly that the more mobile species may be able to reach the outer layers again. Nevertheless a good deal of the skill in mushroom growing seems to consist in the management of the manure during these turnings, and though it is not suggested that the control of animal life is the most important point involved, evidence is not lacking that the fauna is considerably affected. For instance, mites and Phorid larvae, common in fresh manure, have been found to decrease progressively in numbers in samples taken after a number of turnings, until, when the compost was ready to be made up into beds, no flies and very few mites could be obtained from it. This auto-sterilisation process which goes on in the heaps is undoubtedly important, but its effects are as yet incompletely understood. Assuming that the insects and mites are attracted to certain chemotropically active substances in the manure, we may reasonably be allowed to suppose that these will vary a good deal in amount with the successive turnings, and it may well be that while fresh manure will attract certain species, the final compost will not be attractive to them, but on the other hand, it may act in the opposite way or even attract other species. At any rate, growers are wondering if complete sterilisation of the compost can be attained before making the beds or, at any rate, sterilisation of the outer layers where life is most likely to be present. Hence attempts are being made to use steam and insecticidal substances to treat the compost at various stages in an effort to lessen the risk of pest introduction in this way. Sterilisation of the casing soil has already been done commercially, but the object in view has been to suppress fungoid diseases. It has already been pointed out that, as a rule, subsoil free from humus is obtained for casing where possible, and this contains very little animal life. Such supplies of soil are not always available, and when this is the case and surface soil has to be used, sterilisation is probably a wise measure. If surface soil is used, then any of the creatures mentioned before as inhabiting unhygienic buildings may be introduced and, in particular, such soil may possess a large springtail population.

In addition to the definite pests mentioned as known to occur in manure or soil, flies of the family Borboridae must be mentioned, since they often occur in vast numbers on manure heaps and are often suspected by growers. Little is known of their life history, and thus far they have not appeared among the flies bred either from mushroom manure or attacked mushrooms, so that their relation to this investigation is still obscure.

(3) *Those specific to mushrooms.* There is only one group of insects which can lay claim to inclusion in this category, the so-called "mushroom flies" belonging to the genus *Sciara* of the family Mycetophilidae or fungus gnats.

Up to the present we have bred out five species from attacked mushrooms in which the larvae tunnel in a manner similar to that of Phorid larvae, in fact an examination of the tunnels alone gives no clue to the larvae which have been responsible for them. The only reason why one is inclined to place these flies in a category of specific pests is that they have not thus far been bred from manure in any condition before it is actually in the mushroom beds, that is, they are assumed to be attracted to the growing mushrooms themselves or to the active spawn, in fact they ovipost freely upon the mushrooms or upon the soil immediately adjacent. The adults are to be seen making short hopping flights or running actively over the surface of the casing soil. There is a possibility, however, that they may in part enter the beds after these are made and spawned, but before casing takes place; and it seems that the activities both of the Sciarid and Phorid flies during the period which elapses between making the beds and casing, normally about 3 weeks, must be carefully watched. If eggs are laid during this period, we may be justified in assuming that it is the actual growing spawn which attracts the flies, or that manure in that state exerts an attraction because it will sooner or later produce fungus growth of a suitable kind. Sciarid attacks may be found throughout the year when mushrooms are cropping, and by far the largest number of enquiries received relate to damage by these larvae. For this reason work has mainly centred around this group up to the present, and Mr Austin will describe the technique which he has employed and the details which have so far come to light. In all cases cultivated mushrooms alone have been the subject of the work, but we may, with the hope of obtaining useful information, turn to the fauna associated with wild mushrooms perhaps and study the incidence of fly attacks upon them.

II. LABORATORY INVESTIGATIONS.

By M. D. Austin, F.R.E.S.
(*S.E. Agricultural College, Wye, Kent.*)

As already indicated, our laboratory investigations have been concerned mainly with the Dipterous genus *Sciara*. The reason for concentrating on this genus is that the larvae of this and certain other Dipterous genera are major pests of cultivated mushrooms, and few samples of pest-infested mushrooms examined have been entirely free from them. Moreover, in the majority of instances, *Sciara* spp. predominate and provide material for study throughout the greater part of the year. The amount of material available for this purpose has been adequate, but the immediate scope has been frequently limited by the condition of the mushrooms when received. For example, in the case of over-mature mushrooms infested with larvae, it is often impossible to rear successfully adults in this same host owing to its rapid decomposition. In cases such as this the larvae or pupae have to be transferred to other mushrooms. This is of no great importance if the object in view is the determination of the species concerned, but for an accurate study of a life cycle in the case of mushroom pests, it is a drawback as will be explained later, and attempts have been made to overcome the difficulties in this direction by the use of methods to be described.

For the purpose of biological study in the early stages of this work flies were reared from larvae present in attacked mushrooms. Such mushrooms were placed in suitable vessels and in due course the adults emerged, but it soon became apparent that to be of real value in obtaining data relative to the bionomics, the rearing must be made in more controlled conditions. As yet our technique is not fully developed, but the present methods are fairly suitable. In effect miniature mushroom beds are prepared: a suitable box (24 in. × 18 in. × 6 in.) being used for the purpose. Prepared compost is placed within and consolidated to a depth of 3 in.: spawning is carried out at the correct time, and casing soil applied when the mycelium is running freely. To keep the bed at a more or less equable temperature damp moss is lightly placed upon the casing soil, and the whole box covered with sacking. As soon as the buttons appear the moss is dispensed with, but the sacking still used. By these means it is possible to grow a series of mushrooms for use *in situ*, the number of such boxes employed depending on the extent of the work in view. The advantages of the method are that an adequate supply of pest-free mushrooms growing more or less in isolation is thus obtained: individual mushrooms may be conveniently enclosed from above by a glass cylinder with a cellophane top without seriously interfering with the normal growth, and insects in any stage may be introduced in the cylinder and their biology studied within certain limits in more or less natural conditions. A suitable method for a study of this nature is to take a "pair" of flies, *in cop* if possible, and place them within a cylinder as already mentioned, but it is not impracticable to place eggs directly within, on the soil or the mushroom. Of the two methods the latter is possibly the better, inasmuch as a female may die without ovipositing owing to other factors intervening. Even in the event of oviposition occurring, the period elapsing before the eggs hatch much reduces the time available for larval activity in the same mushroom. The disadvantages of the "box method" are that the boxes are in some respects too big for the purpose: larvae can work their way down into the soil or compost and travel to another part of the box: a more serious drawback is that unless larvae attack the mushrooms when they are in the early button stage, the mushrooms develop too rapidly (from our point of view) and soon reach the "broiler" or over-mature stage. For this reason in many cases it is impossible to follow the whole life cycle on or in the same mushroom and, by transferring larvae from one mushroom to another, another factor is involved. Briefly, larvae which are already established within one host have to be introduced to another in which they must of necessity make a fresh entry—a complication possibly upsetting the natural trend of larval activity. Therefore, for the purpose of some portion of our study, recourse was made to another method.

Glass jars half-filled with compost are steamed and after sufficient cooling, flies, larvae or eggs are introduced, the tops of the jars being closed with cellophane. Portions of pure-culture spawn may be used in this medium, but we are not in a position to say that mushrooms will result. It is found that this medium is suitable for rearing flies. An important point was discovered during the use of this method, namely that *Sciara* larvae can live entirely on and in a medium of compost. This means, in effect, that *Sciara* larvae could probably live in a mushroom bed and thrive in the absence of growing mushrooms, and presumably would transfer their attentions to the crop when they encounter the spawn or buttons. If this holds good in normal commercial conditions it may well explain the occurrence of the so-called "sudden" attacks of larvae so frequently met with.

On the whole it may be said that the rearing of flies in these conditions for the purpose of biological study is fairly satisfactory, but a really suitable method for reproducing fly larvae attack in developing mushrooms in isolation has yet to be found.

In passing, it is of interest to note that Thomas (*Ent. News*. XL, 222–5, 1929) in the U.S.A. records the use of bottles of mushroom spawn for pest rearing.

By the use of the methods outlined a measure of success in rearing and breeding flies has been attained and, as already mentioned, the work in this connection has mainly been concerned with flies of the genus *Sciara*. At present five species, namely, *Sciara agraria* Felt., *S. auripila* Winn., *S. vivida* Winn., *S. umbratica* Zett. and *S. fenestralis* Zett., have been successfully reared from larvae present in attacked mushrooms.

Briefly the life cycle of a *Sciara* is as follows: the white translucent eggs are laid in strings of from four to twelve or singly on the casing soil or on the mushrooms themselves. Occasionally eggs may be found on the caps, but they are more frequently laid at the point where two mushrooms converge and meet at the base. The old cut stumps also offer a suitable situation much favoured by the flies for oviposition. However, generally speaking, the majority of the eggs are laid on the soil. These hatch within 4–7 days and give rise to minute larvae approximately 0·9 mm. in length. The larvae have black head capsules, and usually are readily distinguishable from other fly larvae associated with mushrooms. The newly-hatched larvae enter the mushrooms at the base at about soil level and work their way upwards. Feeding extends over a period of from 15 to 20 days and the fully-grown larvae may be up to 10 mm. in length. From 2 to 3 days are often spent by the larvae of certain species in preparing a cocoon, and the pupation period is from 8 to 14 days. Pupae are found in a variety of situations, between the gills of the mushrooms, in the larval tunnels and, more frequently, in the soil. The typical life cycle therefore takes a minimum of 29 days and a maximum of 44 days to be completed. Certain factors such as presence of suitable food and temperature may play important parts in limiting or lengthening the duration of the cycle. In the case of *S. fenestralis* a short note on its biology has been recently published (*Journ. S.E.A. Coll.* XXXII, 59–62, 1933). The adults copulate on the day of emergence and oviposition usually takes place within 2–3 days. Females have been observed to lay up to thirty eggs but, as these were laid in captivity, this figure may possibly be exceeded in normal conditions. Short jumping flights are characteristic of the *Sciara*, and they seldom seem to leave the beds unless attracted to light. The males are easily distinguished from the females on account of the comparatively large claspers associated with which, incidentally, characters of diagnostic importance are to be found.

It is of interest and certainly of importance to note that with such a comparatively short life cycle it is possible and not unusual for two, three or more generations of flies to occur during the "life" of a mushroom bed, the implication being that it is imperative to control these pests early on, especially as there is often an overlapping of generations and consequently a continuous horde of larvae causing damage.

As agents in the spread of mushroom diseases the rôle of these and other flies is uncertain, but in all probability they are active vectors. It has been observed that mites often become attached to these flies and thus spread from place to place: *Tyroglyphus mycophagus* Megnin has been noted as being spread in this manner.

Mr Jary has already touched upon the subject of damage, and it remains only to indicate that there are several types. The most obvious is that caused by the larvae tunnelling up the mushroom stems: here the damage is in the form of extensive galleries or tunnels, and these tunnels often extend into the caps. As many as fifty larvae have been found in one mushroom; however, it is as well to point out that a few (three to five) are sufficient to cause serious damage. The larvae are also responsible for severing the mycelial strands from the stalks, and are suspected of damaging the spawn before it has commenced to "run" or during the time when it is "running." This last form of damage is insidious, inasmuch as unless one knows larvae are present, serious damage may be caused and a poor or no crop result. The spawn may itself be blamed for the lack of crop in such a case.

It is a simple matter to speak of *Sciara* attacks, but in practice other complications set in. For instance, the larval tunnels are frequently infested with *Collembola* spp. and mites which often damage the mushrooms still further and, moreover, other fly larvae may also be present.

This then is our present contribution to the study of *Sciara* spp. as pests of mushrooms: the biology of most of the species mentioned has still to be checked up, so it is now convenient to mention briefly a few notes concerning other fauna associated with cultivated mushrooms.

As an integral part of the preliminary investigation a special study was made of this fauna with reference to which two notes have been published in the *E.M.M.* LXIX, 16–19, 132–134, 1933, in which flies other than *Sciara* have also been noted.

The more important of these belong to the family Phoridae, and the larvae of one, *Aphiocheata albidihalteris* Felt., have been frequently met with. These larvae cause serious damage to mushrooms, more especially in the summer months in weather such as obtained during the past year. The biology of species of Phoridae is now being investigated. The larvae are often found in the compost before and after the beds are made up, and mushrooms often contain up to 100 larvae. Outdoor beds at Wye suffered a very heavy attack during the past year, whereas indoor beds but a few yards away were pest-free to a marked degree. An examination of the beds, their environment and the data available on the past history indicated that the original infestation occurred during the period between spawning and casing. Incidentally it is this period which must be closely watched in the future in any study of the economy of the beds. The beds in question, as is usual with outdoor beds, were straw-covered, and it was found that pupae were present and attached to this covering. Consequently when control measures were initiated on the beds, the infested straw was repeatedly removed and replaced, and success was not obtained until control measures included infested straw treatment or its absolute removal. Another point of some interest is that it is not unusual to have a very high degree of pupal parasitism, although it has been observed that parasitism is mostly in evidence after the Phoridae have obtained a good footing in the beds. The parasite mainly concerned is the Braconid, *Aspilota concolor* Nees.

Cecidomyiidae larvae have been found heavily infesting mushrooms in some localities, but apart from the fact that these larvae have caused serious damage somewhat similar to that caused by other fly larvae, little can now be said of their biology. Repeated attempts have been made to rear them to maturity but without success. It is of interest to note, however, that the majority of such infestations were recorded during the winter months. There is of course at least one known Cecidomyiidae pest of mushrooms, namely *Mycophila speyeri* Barnes, noted by Barnes (*E.M.M.* LXII, 89–92, 1926) and Speyer (*Expt. and Res. Sta. Cheshunt, Ann. Rpt.* 1926, 56–7, 1927).

Another fly often occurring in mushroom houses is *Drosophila funebris* F., more commonly known as the "vinegar fly"; this also has been reared from larvae-infested mushrooms during this investigation. The larvae were present in huge numbers in badly damaged mushrooms, but our evidence leads us to consider that, as Drosophilids usually oviposit in putrefying matter, mushrooms in a condition of breakdown offer a suitable medium. To be more explicit, *Sciara* and Phorid attacks are usually characterised by well-defined tunnels in otherwise sound tissues, whereas with *Drosophila* the mushroom tissues are in a more or less fluid state, and, literally speaking, the larvae ooze in and out.

The Borborid, *Limosina ferruginata* Stck., has frequently been taken from around compost heaps and also from an established bed. The biology of this fly, as far as we are aware, is unknown, and certainly its position as a factor in the economy of mushroom beds is uncertain. At present it can only be stated that this fly has not yet been reared from larvae-infested mushrooms and manure examined during this investigation.

For the sake of completeness of this account of the fauna commonly met with during the past two years, it should be mentioned that various species of *Collembola* and mites often occur, and that an eelworm, *Rhabdites teres* Schnr., has been noted as apparently causing damage in mushrooms.

Finally, to give some idea of the amount of material received over the past year alone, we mention that specimens and queries have been received from Kent, Sussex, Surrey, Suffolk, Northamptonshire, Hertfordshire, Gloucestershire, Lincolnshire, Hampshire, Wiltshire, Cambridgeshire and Herefordshire, and also from Wales and Scotland, involving over fifty separate enquiries—substantial evidence that there is pressing need for further knowledge of mushroom pests and their control.

With reference to the work on the control of these pests, both individually and collectively, we have been limited in scope on the one hand by the nature of the material received, and on the other hand by the methods of culture peculiar to the industry. Naturally, although we constantly receive attacked mushrooms, infested manure, etc., the order, family or species of pests concerned are not necessarily the same. As to the cultural aspect of the problem it is not yet possible to ask a grower to allow one to experiment on an established mushroom bed in the same way as one approaches, say, a fruitgrower for permission to use a block of trees. Thus, although the whole problem of pest control needs attacking, our work has mainly been restricted to laboratory tests on a small scale.

In this connection pyrethrum, nicotine and derris dusts and sprays have been used and at present we are inclined to advocate the use of a nicotine spray for fly control, especially *Sciara*. An important point to be considered in relation to direct control measures involving the use of sprays and dusts is the "damage factor." Materials have been tried out with this point always in mind. In the case of the "white" variety of mushroom, staining of the cap may easily occur with the use of oil sprays, and at least one case has been observed where pyrethrum dust has left an unsightly deposit on the caps.

Moreover as temperature and humidity play a considerable part in the economy of mushroom culture, the effect of these and other factors must be considered. To study adequately the interrelation of these and the incidence of pests, a special building is a necessity and consequently little has yet been done in this direction, the investigations here described being in the nature of a preliminary study designed to facilitate further work.

REVIEWS

Recent Advances in Cytology. By C. D. Darlington. Pp. xviii + 559, 8 plates. London: J. and A. Churchill, 1932. 18*s.*

This volume seems a little out of place in the "Recent Advances" series, many of which, with all due deference to their authors, are scissors and paste work. True, the book contains a résumé of cytological investigations published during the last ten years or so but, at the same time, it is a serious and constructive work and, in many ways, an original contribution to biological literature. It is not as its title implies a treatise on cytology but, as J. B. S. Haldane says in his foreword, "a treatise on karyology," and "in karyology it marks the beginning of a new epoch, the transition from an essentially descriptive to a largely deductive science." The author describes his book as consisting "of three elements: an introduction for the student who is a beginner, a résumé for the research worker who requires classified observations, and a theoretical treatise, in which my own views are developed, for the general biologist."

The book contains sixteen chapters and four appendices. Chapter I is a general introduction to the study of the nucleus, cell and organism, reproduction and heredity. Chapters II and III are devoted to mitosis, Chapters IV–VI to meiosis and Chapters VII and VIII to polyploidy. This portion of the book, although fairly stiff-going and discussing much controversial matter, is, on the whole, straightforward and is recommended by the author to the student as "a guide to his early observations." A beginning student who survives thus far may surely count himself more than weaned and proceed with some degree of confidence; he will certainly no longer be a beginner. Chapters IX and X deal with the chromosomes in heredity, Chapters XI–XIII with normal and abnormal meiosis, Chapter XIV with permanent diploid hybrids, and Chapter XV with apomixis. If the student has really assimilated these chapters, and still survives, he will know more about cytology than a great many biologists. Chapter XVI, perhaps the most important chapter in the book, deals with the evolution of genetic systems and is an interesting contribution to our ideas on such fundamental biological problems as the sources of variation, the nature of species and the principles of evolutionary change. The author's ideas are not, of course, entirely new, but they have rarely been put in a more succinct way or based on so much direct evidence. This chapter will be read with interest by every serious student of evolutionary biology; it will certainly cause many older and more experienced biologists to shake their heads and one may safely predict that it will stimulate no little research and controversy. The author's main conclusion may perhaps be extracted as follows: "Variation...is discontinuous...and random. Yet from the few gene changes that prove satisfactory all organic variation is derived." The appendices concern (1) cytological interpretation, (2) recent improvements in technique, (3) an interesting glossary, and (4) a bibliography containing some 900 references, mostly botanical. The book opens with a very full table of contents and closes with a good index.

The author has worked through a mass of cyto-genetic literature and has attempted to clarify the data by the deductive application of certain fundamental hypotheses which he has formulated and which are essentially based on the relation of chiasmata to mitosis and meiosis. Whether or not one accepts the author's interpretations, one cannot help but admire the masterly way in which he has analysed and marshalled an almost overwhelming amount of data into a perfectly logical scheme in support of his views. The work is impressive and convincing, and the book is not only a major contribution to English biological literature but is something of a landmark in its particular region. Every serious student of cytology and genetics will find it indispensable.

W. B. BRIERLEY.

Recent Advances in Plant Genetics. By F. W. Sansome and J. Philp. Pp. x+414. London: J. and A. Churchill, 1932. 15*s*.

In this book the authors have attempted to summarise the chief advances made in plant genetics during the last decade. In this period the subject has developed more rapidly than most other branches of biology, and it has been beyond the capacity of any save a few research workers in genetics with access to first-class libraries to keep up-to-date with the welter of investigations which have been published. From time to time useful text-books of a general nature have appeared but these have drawn their illustrations largely from zoological sources, whilst the more monographic botanical treatises have dealt intensively with only one or another restricted field of plant study. All workers in biology have felt the need for some such survey as the authors have produced, but few have had the courage to consider facing up to such a task. Further, during the last ten years, the most important developments in plant genetics have derived from the experimental study of polyploidy, a phenomena apparently almost peculiar to plants but of fundamental significance for the general chromosome theory of heredity. These botanical data seem to have been largely unknown to zoological students, and it had become essential that an adequate survey and synthesis be made of them so that they could be considered in relation to animal data.

It is, of course, quite impossible in a small volume to cover the whole field of plant genetics, and the authors have wisely adopted the course of selecting certain aspects for more extensive consideration, whilst subordinating others in order to give what in their judgment is the most valuable perspective. It is unfortunate that this has meant summary treatment of such important questions as sex in plants, chimaeras and the mathematics of inheritance and populations, but references to these subjects are given in the bibliography and fortunately, in recent years, they have received more or less adequate treatment elsewhere. Another issue which the authors barely mention is the artificial induction of mutation, perhaps the most potentially significant discovery in modern biology, and the important papers on this question, published by Stadler in *Scientific Agriculture*, 1931, are not included in the bibliography.

The first nine chapters of the book deal severally with inheritance in diploids, the chromosome theory of heredity, the constitution of the factor, the origin of polyploidy, autotetraploids, allopolyploids, euploids and aneuploids, structural hybrids and, finally, interspecific hybridisation. These chapters consist of good, straight, technical writing and show throughout a grasp and insight and a critical acumen which only comes from intimate personal experience of research in the special field dealt with.

The last chapter, x, stands rather distinct from the rest of the book, although in many ways it is the climax to which the preceding chapters naturally lead. It is not, as are the previous chapters, a résumé of other people's work but it contains the authors' own interpretation of these facts and the conclusions they draw from their survey. The last few pages of the chapter are devoted to a consideration of the species problem and one hopes that some day the authors may expand this into a separate work.

The volume is illustrated by 56 figures and 42 tables: it opens with an appreciative foreword by Sir Daniel Hall and terminates with a most useful bibliography of some 1200 references, and an adequate index.

In the preface the authors hope that the book will "be useful to the honours students in genetics" although they have "intended it primarily for the teacher, the research geneticist (applied and purely scientific) and the general biologist." For any one of these it is a book, not so much for library consultation as for personal possession, a book to be read and marked, and it is, therefore, a pity that it could not have been published at a lower price.

W. B. BRIERLEY.

The Apple. By Sir A. Daniel Hall and M. B. Crane. Pp. 235, 11 figs. and 12 plates. London: Martin Hopkinson, Ltd., 1933. 10*s*. 6*d*. net.

In the preface which is signed by Sir Daniel Hall only it is stated that "this book is written for the practical man, and is an attempt to put him in touch with recent developments in the practice and science of apple growing." To do this fully would require a much larger volume than the present one and the plan adopted by the authors is "to describe standard practice and to give references to the sources of fuller information."

Chapter I is an interesting but brief account of the history of the apple both in regard to its origin in cultivation and the development of apple growing in this country. The next four chapters deal with the genetical study of the apple and the remaining eight with apple cultivation. Chapter II begins with a botanical description of the structure of the apple fruit and then plunges suddenly into an account of nuclear structure and polyploidy. Chapters III–V are devoted to variation and fluctuation in the apple, to fertilisation, sterilisation and incompatibility in relation to chromosome content and finally to apple breeding and seedling raising. Many growers, unaccustomed to genetic shorthand and terminology, will find these four chapters heavy going. In his interesting preface Sir Daniel Hall effectively disposes of this criticism: which however, whilst it may encourage the practical man, will not make the chapters any more comprehensible to him. Many a harassed grower will frown when he reads (p. 91) that a particular factorial combination "will only occur once in thirty-six times in a heterozygous auto-tetraploid with four homologous chromosomes," and if he does not close the book here he may do so at the bottom of p. 96; which would be a pity.

Chapter VI is a useful but rather slight consideration of soil, manuring and cultivation, and one may note a type-slip in the heading of p. 113, and two pages later, the use of the obsolete term "fungoid disease." The next four chapters deal respectively with the grass orchard, stocks and propagation, lay-out, and pruning, whilst Chapter XI contains a very brief account of pests, diseases and spraying. Chapter XII, the longest in the book, contains a description of selected apple varieties, and Chapter XIII, the shortest, is a brief discussion of the picking and storage of apples. The book closes with a useful bibliography, a glossary of terms and a good index. It is most attractively produced and well printed and is illustrated by twelve excellent plates and eleven very clear text-figures.

This little volume brings together a surprisingly large number of data drawn chiefly from investigations carried out at the John Innes Horticultural Institution and the Long Ashton and East Malling Research Stations, and the whole book is illuminated by practical wisdom and wide experience. It is difficult to know for whom the book has really been written. It is stated that it is intended for the "practical man," but the practical man will certainly want far more practical detail than the authors give, and can get this more easily from the excellent publications of the Ministry of Agriculture. Also the practical man will not find much opportunity, even if he had the wish, to consult "Blomefield, R. 1775. *History of Norfolk*," or "Kobel, F. 1927. Zytologische Untersuchungen an Prunoideen und Pomoideen. *Arch. der Jul.-Klaus-Stift.*, 3, 1." or "Winkler, H. 1914. Die Chimärenforschung als Methode der Experimentellen Biologie. *Sitzber. Phys.-Med. Hes. Wurzburg*, 1." For the student the book is not nearly complete enough, and in any case falls between quite a number of stools. One cannot help feeling regret that whilst the authors were about it they did not produce a book on "The Apple" more comparable to the senior author's book on "The Tulip." So far as it goes however the book fulfils its aim of putting a reader in touch with recent developments in the practice and science of apple growing, and much of this knowledge is quite indispensable to an understanding of the rationale underlying apple behaviour and the cultivation of the crop.

W. B. BRIERLEY.

Elements of Agriculture. By the late W. FREAM, LL.D. Ed. by Prof. Sir ROWLAND H. BIFFEN, F.R.S. Pp. viii + 720, 132 figs. and 15 plates. London: John Murray, 1932. 10*s*. 6*d*. net.

Fream's *Elements* is perhaps the most widely known single book in agricultural literature, and the issue of a completely new edition is an important event in the history of the science. First edited and mostly written by Fream in 1902 at the request of the

Royal Agricultural Society of England it ran in this form through seven editions. An eighth edition edited by Ainsworth Davis appeared in 1911 and ran through four editions. The present edition edited by Biffen (who undertook the task on the death of T. B. Wood) has been prepared by the staff of the Cambridge School of Agriculture. Each subject was allocated to the appropriate member of staff with instructions to include in his portion matters suitable for students at agricultural colleges and schools, and for farmers who wish to gain an all-round general knowledge of agricultural science. Each writer has attempted to produce a readable story giving references for further study, to relate his particular problems to cognate issues and to show their bearing on agricultural practice. It may be noted in passing that references are not appended to certain of the chapters, and that in other cases they are by no means the best that could have been advised.

The ground plan of the earlier edition has been maintained, the book opening with a consideration of soils and implements, passing to plants and concluding with animals. The chapters run into each other fairly smoothly and naturally. The soil and farm implement chapters lead to questions of tillage, drainage and manuring. A discussion of sequence of cropping then leads to a general treatment of farm crops, the cultivation and harvesting of cereal, pulse and forage crops, seed rate and sowing, and weeds. Grasses are next treated, leading naturally to grass-land questions, ensilage and haymaking. This is followed by two rather isolated chapters, one dealing with hardy fruit culture and market gardening and the other with plant diseases. The two biological portions of the book are linked by a chapter on plant and animal breeding. This is followed by a general consideration of the structure, functions and nutrition of the animal body proceeding to the special treatment of the breeds and management of horses, cattle, sheep, pigs and poultry. Animal production then leads naturally to dairy products and animal hygiene. The last chapter is an isolated one on insect pests.

In the new edition there has been very considerable alteration and redistribution in the actual subject-matter, this being less marked in the soil chapters than in those dealing with animals and plants. The omitted matter is largely of an elementary or introductory nature, *e.g.* Chapters IX–XI of the eleventh edition consisted largely of an elementary introduction to the botany of crop plants, and this knowledge is rather taken for granted in the new edition. Similarly in the twelfth edition a good deal of elementary vertebrate zoology has been condensed or omitted from the animal husbandry chapters. In the above cases this is a desirable course, but its application in certain other chapters is more questionable. Thus the old edition contained a useful chapter on harmful and beneficial animals, which included in its purview mammals, birds, insects, arachnids, myriapods, round and flat worms and protozoa. The corresponding chapter in the new volume confines itself to insects with casual notice of myriapods and arachnids: *Aphelenchoides* and *Anguillulina*, moles and musk-rats, rabbits and rooks are not mentioned. On the other hand, a tremendous amount of new material has been incorporated in the new edition—new chapters on the sequence of cropping, the cultivation and harvesting of cereals, the cultivation of pulse and forage crops, seed rate and sowing, the systematics and biology of the grasses, and animal hygiene. Of course a certain amount of the older matter of these chapters was to be found scattered through the various chapters in the earlier editions, but in the present volume it has been brought up to date and greatly amplified. In addition, however, to these new portions all the chapters in the book have been rewritten and in most cases bear little resemblance to their predecessors. Moreover, an increase in the relative size of the biological portion, more especially in the part dealing with animal production, has brought the general perspective more into line with modern views on agricultural research and practice, although a somewhat surprising omission is any reference to agricultural economics.

It is not, of course, possible here to enter into a detailed criticism of a book covering so wide a field, but certainly every reader with special knowledge will note sins of omission and commission. Some of these concern the general matter of a chapter, *e.g.* Chapter I on the soil, which is neither up-to-date nor balanced in relation to the soil microbiological aspects, or, on the other hand, they may be of a mere incidental nature as in Chapter XVI on plant diseases where there is a heading "Interplant mycelium," the use of the word "fungoid" or the description of the reproductive bodies of *Synchitrium endobioticum* as "resting spores." On the whole, however, the book is remarkably free from actual errors and misprints and, although certain chapters are of very pedestrian quality, others are quite unusually good. An unevenness in quality is almost inevitable in a book written by eighteen authors, but a little more editorial severity might have improved matters.

There are two differences that will strike everyone who compares the present with the last edition. One is the change in format of the book from a squat and somewhat badly printed tome to a really graceful volume, and this change, incidentally, has allowed for an increase of about 100,000 words in the size of the book. The other is the fact that the number of illustrations has been cut down by one half. Some of the old favourites are left, such as the wonderful Cotswold soil-profile, the beautiful ploughing furrows, the famous spanner and threshing machine, the cattle, sheep, and pig portraits, the grasses and the insect pests, but others such as the fowl portraits and nearly all the crop and weed illustrations have disappeared. On the other hand, there are numerous new pictures of more modern farm implements, of grass structure; new figures, with one exception, throughout the plant disease chapter; a percheron portrait; a plate of breeding beef and dairy types of cow; many illustrations of butchers' cuts reminiscent of Mrs Beeton; battery breeders for fowls, and a series of interesting pictures showing changes in proportion with animal growth.

There can be no doubt that Fream's *Elements* will deservedly acquire a new lease of life, the new edition more than replacing the older one. As a single comprehensive book on agriculture the present volume has no equal and, at its price of 10*s.* 6*d.*, is remarkable value.

W. B. BRIERLEY.

Plant Parasitic Nematodes. By T. Goodey. Pp. xx+306. 136 figs. London: Methuen and Co., Ltd., 1933. 21*s.* net.

For long enough the plant-parasitic nematodes have been a sort of no-man's-land invaded or avoided by botanist and zoologist alike, but really cultivated by scarce a handful of devoted workers. Among these, in this country, the author stands preeminent and this book will deservedly enhance his reputation. The ravages and grave potentialities of nematodes are obvious to every one concerned with the growing of plants, and every applied biologist and crop cultivator, sooner or later, finds urgent need for an authoritative source of information from which he can learn of their bionomics and control. If one has access to a good library one can consult the works of Goffart, Rostrup and Thomsen, Sorauer's *Handbuch*, incidental chapters in Heald or lesser manuals of plant diseases or more detailed and restricted memoirs in research journals, but the need has long been recognised for a single treatise bringing together the salient facts concerning all the known plant-parasitic nematodes both as specific organisms and as producers of disease. This task Dr Goodey has achieved, and in so doing has earned the gratitude of all applied biologists.

Chapter I is a very useful introduction, beginning with an account of the general structure of a nematode, proceeding to a description of up-to-date methods of study, and concluding with an account of the systematics of the organisms. The author is strict in his nomenclatural usage, and has no hesitation in degrading old established and widely accepted names if he can find one with prior claim. Thus the familiar *Tylenchus* becomes *Anguillulina*, *Isonchus* becomes *Aphelenchus* and *Pathoaphelenchus* becomes *Aphelenchoides*; *Tylenchus hordei*, of ill-fame, becomes *Anguillulina radicicola* and *Heterodera radicola* becomes *H. marioni*: which is all, no doubt, very accurate but very confusing to the botanist or zoologist who does not happen to be a technical helminthologist.

Chapters II–VII deal in a very clear and straightforward way with the obligate eel-worm parasites, beginning with *Anguillulina* and passing to *Tylenchulus*, *Heterodera* and *Aphelenchoides*. Chapter VIII is devoted to nemas of doubtful pathogenicity, saprophytes and predators, whilst the last chapter is an all too brief discussion of biologic races in plant-parasitic nematodes. All the parasitic species are dealt with in the same logical order—name and synonyms, brief historical account of investigations dealing with the species, morphology including dimensions of female and male with formulae, descriptions of adults, larvae, and eggs, bionomics including life history, symptoms and pathology, host-range, geographical distribution, and finally control measures. In addition to this general schedule of treatment the accounts of better known species usually contain additional sections dealing with the relation of the organisms to soil and meteorological conditions, persistence, methods of dispersal and natural enemies. Where the species occurs on several hosts there is included a list of the recorded hosts (which in the case of *Heterodera marioni* runs to some 600 names, both scientific and popular name being given) and there are often special discussions of the disease and control measures for the different important crop plants.

The book is written in a condensed yet very readable style, containing in compact form a great amount of information, and being illumined throughout by the author's own researches and unique experience. Each chapter is followed by a selected list of references, care having been taken to include such as contain more comprehensive bibliographies. These lists are very up-to-date, even 1933 publications being cited. Special mention must be made of the illustrations, many of which are the very beautiful and accurate line-drawings which we have long associated with Dr Goodey's work. The book opens with a foreword by R. T. Leiper, contains a full contents bill and list of illustrations, and closes with an adequate subject and author index: the only useful thing missing is a host index. One may note here that occasional misprints occur in the spelling of plant names throughout the volume, and also that in a second edition Figs. 24 and 32 might well be replaced by more adequate illustrations.

Reading through Dr Goodey's book it becomes abundantly clear that problems of nematology impinge on plant physiology and pathology, plant genetics and the more general problems of plant protection, applied entomology, and the microbiology, chemistry and physics of the soil. Workers in all these fields will find in the book suggestions of interesting and important problems needing investigation. The last chapter on biologic races is perhaps of special interest and will cause many readers to give attention to this phenomenon. The problem of biologic races, leading as it does straight to the problem of the nature and constancy of the species, opens up theoretical issues of fundamental importance in biology, whilst the immediate and practical needs of disease incidence and control imperatively demand its study. The author could scarcely have found a better note on which to end his book.

W. B. BRIERLEY.

Phytopathological and Botanical Research Methods. By THOMAS ELSWORTH RAWLINS. Pp. ix+156. London: Chapman and Hall, Ltd., 1933. 15*s*. 6*d*. net.

In compiling this book the author has searched an enormous number of publications in biology and the cognate sciences and extracted such methods as may prove of use to the botanist and, more especially, the phytopathologist. The work is divided into three parts. Part I, four pages long and purely introductory, is headed "Choosing and Planning a Research Project," and is a queer jumble of omissions and commissions, counsels of perfection and extreme naïvety. It contains for example "a list of indexes and other papers which are very useful to the phytopathologist in locating literature related to his proposed research." Among these is Dorfler's *Botaniker-Addressbuch* published in 1909 and entirely obsolete whilst the *International Address Book of Botanists* issued in 1931 is omitted; various American abstracting journals are given, but the principal German and French ones are excluded, and so forth.

Part II, which runs to 94 pages and is more than half the book, is headed "Experimental Methods," and consists of four chapters dealing respectively with microscopic methods, including a very good section on microchemistry, culture methods, virus studies, and miscellaneous experimental methods. One may say at once that this is perhaps the most useful account of phytopathological technique that has been published. Great care has obviously been taken in the selection of the methods and, usually, the several stages of any operation are so clearly and simply described that a beginner could follow them. Most of them seem to have been personally tested by the author, who often suggests improvements in the original schedules but, from time to time, one gets the impression that the author is writing from book knowledge only and, occasionally, there are such sentences as: "Lime pith is said to be more rigid...," etc. On the other hand this portion of the book is sometimes tantalising, *e.g.* the section "Growing Plants Under Sterile Conditions" contains the following: "Numerous methods have been described for sterilising seeds and growing plants under sterile conditions: (485), (514), (563), (577), (581), (638), (645), and (650)"—and that is all!

Part III, with the somewhat grandiose heading "Interpretation of Experimental Results," is four and a half pages long, attempts to "present methods which are applicable to the analysis of quantitative experimental results" and simply—very simply—deals with probable error and standard deviation. It would have been an improvement if the author had treated the "Interpretation of Experimental Results" as he treats "Growing Plants Under Sterile Conditions." The remainder of Part III is occupied by a bibliography and an index. The bibliography contains over 1000 references classified alphabetically under authors' names in six sections—histology and cytology, microchemistry, culture methods, viruses, miscellaneous methods and interpretation of experimental results, and it is a valuable part of the book. A very useful reference omitted from the last section is Tippett's *The Methods of Statistics* (1931) which is written for workers in the biological sciences.

The task of an author in selecting the material for such a book as this is not to be envied, since every investigator has his own favourite methods and is sure to complain if one or another is not mentioned. The omissions are obvious and important, but most of them are covered by the bibliography. On the other hand one must recognise that the author has carried out his positive selections very judiciously, and has brought together in compact form a great number of very useful methods. The book is, in fact, almost indispensable to every working phytopathologist, and general botanists will find it a valuable compendium. It is well printed—with occasional misprints—and, being strongly bound with a waterproof cover, will stand up to ordinary laboratory usage.

W. B. BRIERLEY.

Vol. XXI, No. 2 May, 1934

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VOLUME XXI MAY, 1934 No. 2

SOME FACTORS AFFECTING THE INACTIVATION RATE OF THE VIRUS OF TOMATO SPOTTED WILT

BY J. G. BALD, M.AGR.SC.

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(With 3 Text-figures.)

IN a previous paper (2) it was shown that the virus of tomato spotted wilt became inactive after about 3 hours[1] when the expressed infective juice containing it was left standing at room temperature; also (5) that the inactivation followed a definite course when examined quantitatively by the primary lesion method, and that it could be represented by a logarithmic curve of a type corresponding to that of a unimolecular chemical reaction. It was suggested that the loss of virulence with time was possibly due to a chemical reaction of the nature of an oxidation.[2] This idea has been followed up by further tests, which include the addition of oxidising and reducing agents to the inoculum, and the results are reported in this paper.

Allard (1) found that small quantities of hydrogen peroxide added to the expressed sap of mosaic tobacco plants destroyed the peroxidase without affecting the virus, but that larger quantities of hydrogen peroxide destroyed the virus also. Johnson (3), in studies on the over-wintering of tobacco mosaic virus in the soil, treated some extracts with air and oxygen and found a considerable reduction in the activity of the virus as a result of these treatments. No other work on the effect of oxidising or reducing agents on plant viruses appears to have been published.

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[1] Subsequent experiments have shown that the time taken for complete death of the virus may vary, at a temperature of about 20° C., from less than 2 to more than 5 hours. It will readily be seen from the form of the inactivation curve that the initial concentration of virus present in the juice will affect considerably the time taken for complete death, and the work presented in this paper would suggest that factors affecting the composition of the plant juice may also exert an appreciable influence.

[2] This implies that the number of lesions bears a linear relation to the number of virus particles, of which there is now some evidence.

Zinsser and Seastone (6) in 1930 found that herpes virus which was kept in open tubes was inactive after 17 and 24 days, whereas virus sealed with the reducing agent cysteine hydrochloride was still active after these periods.

Perdrau (4) in 1932 found that broth filtrates of the virus of herpes could be inactivated by oxygen and subsequently reactivated by a process of reduction. The phenomenon was not a constant one, however, since it was observed in only four out of thirteen filtrates, but the experiments could be repeated on filtrates which had once proved vulnerable to oxygen.

The great advantage of work with plant viruses such as tomato spotted wilt is that the course of the reaction can be followed so well by the primary lesion method, and curves can be plotted showing the changes in concentration of the virus with time.

METHODS.

In most experiments involving the comparison of two samples of virus the alternating half-leaf method described in our previous paper (5) was used. The test plant was Blue Pryor tobacco. On account of the rapid inactivation rate of spotted wilt virus it was found advisable, when inoculating a number of replicates, to inoculate each leaf with the two test samples immediately. This is a slight variation of the method previously described for tobacco mosaic, in which the full number of replicates (half-leaves) was done with one sample first and then the opposite half-leaves with the other.

In some experiments, particularly when several treatments such as strengths of reducing agents were being compared, whole leaves were inoculated on a definitely randomised plan, frequently a Latin square, determined before the experiment by the dealing of cards. In this way data were obtained for analysis in which allowance was made both for plant variation and progressive change in strength of the various batches of inocula with time. Fuller details of arrangement are given in describing certain of the experiments. In all cases the leaf or the half-leaf was taken as the unit, and a reference to "sixteen replicates" means that the tests were made on sixteen leaves. These may have been comparable leaves on sixteen separate plants, or two nearly comparable leaves on each of eight plants.

In the experimental part of the paper the word virus is frequently used for the infective sample of juice itself. In all cases the inoculum was obtained by crushing in a mortar the top leaves of diseased tomatoes that showed the bronzing symptoms of spotted wilt, and expressing the juice through muslin. A 1 : 20 sample of virus means 1 part of this infective juice in 19 parts of distilled water.

All experiments described have been repeated at least once with essentially the same results.

EFFECT OF STIRRING ON THE VIRULENCE OF INOCULUM.

In samples of virus stirred by a spatula during inoculation inactivation seemed more rapid than in a bulk sample of the same virus left undisturbed. To determine whether the difference was real a 1 : 20 sample of fresh virus was divided into two lots of 30 c.c. each; one (A) was left standing in an unplugged flask, while the other (B) was used to inoculate sixteen tobacco leaves on both sides. The inoculum B was stirred no more than is usual in the process of dipping the spatula. This preliminary inoculation served both to give the extra stirring to sample B and to provide a check on the technique employed. Sample A was divided, and one part was compared with B by the half-leaf method on sixteen leaves, and after a short interval a further comparison was made between sample B and the undisturbed remainder of sample A. The results are shown in Table I. It will be seen that the stirred sample B lost its virulence much more rapidly than A, the figures showing the differences to be highly significant.

Table I.

Comparative virulence of spotted wilt inocula stirred and standing exposed to air. Numbers of primary lesions.

Time of inoc. (min.) after expressing juice	Sample	Mean no. of lesions	Mean difference	Standard error	No. of replicates
2–8	B (stirred)	102·8	4·3	7·3	16
	B ,,	98·5			
14–21	A (not stirred)	65·8	35·7*	8·7	16
	B (stirred)	30·1			
23–31	A (not stirred)	45·1	39·3*	5·1	16
	B (stirred)	5·8			

* Differences significant.

BUBBLING AIR AND NITROGEN THROUGH INOCULUM.

Increased aeration seemed a likely reason for the destructive effect of stirring on the virus, so the results of bubbling air and nitrogen through the inoculum were investigated.

A 1 : 20 sample of virus was divided into three equal parts, one of which was left standing in an open tube and is hereinafter called the control. Air was drawn rapidly through a second part, the air first being saturated with water vapour to prevent a fall of temperature in the inoculum. Nitrogen was passed through the third part to exclude free oxygen. The nitrogen used was bubbled through alkaline pyrogallol, normal HCl and water before being passed into the inoculum, and may be considered free from all but the merest traces of oxygen. For the first 10 min. it was bubbled briskly to displace the oxygen dissolved in the inoculum, and later more slowly. All three tubes of inoculum were kept in a siphon water bath which held the temperature between 22 and 22·2° C.

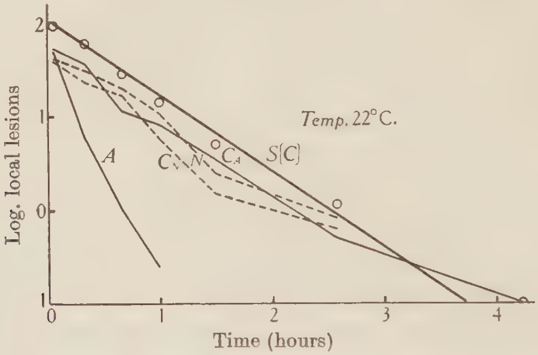

Fig. 1. The effect of bubbling air and nitrogen through spotted wilt inoculum; *A*, aerated inoculum; C_A, control for aerated inoculum; *N*, nitrogenated inoculum; C_N, control for nitrogenated inoculum; *S*(*C*), straight line fitted to points representing the sum of the numbers of lesions for the two controls at each sampling.

Aliquots were withdrawn at intervals by means of pipettes, and compared by inoculation on tobacco leaves. Each set of equivalent time samples was inoculated on a separate batch of plants as uniform as possible. An attempt was thus made to increase the significance of differences between the samples compared at any one time at some sacrifice of uniformity in the batches used for successive time samples. This is reflected in the form of the curves obtained (Fig. 1), the low point in the nitrogen curves at time 90 min., for example, being due to a somewhat poorer batch of tobacco plants used for that test.

The two treatments were compared separately with the control by the alternating half-leaf method. Each comparison *AC* (aerated on the left side, control on the right), *CA*, *NC* (nitrogenated left, control right) and *CN* was considered as a separate experimental unit, and the four were randomised as a Latin square. The leaves were inoculated in the order given by this arrangement. By such means allowance was made for any systematic differences between inoculation of left and right half-leaves and for the time gradient due to continuous inactivation of the virus. Separate portions of the control inoculum were used to compare with the nitrogenated and aerated samples so that no lot of inoculum would be stirred more than another during inoculation.

Table II.

Effect of bubbling air and nitrogen through spotted wilt inoculum. Mean numbers of primary lesions on eight replicates.

Time (min.)	Mean no. of lesions		Mean difference	Mean no. of lesions		Mean difference
	Aerated	Control		Nitro-genated	Control	
3	49·1	52·3	− 3·2	40·4	39·0	+1·4
20	5·9	35·8	−29·9*	30·5	22·9	+7·6*
40	1·0	11·3	−10·3*	19·8	16·3	+3·5
60	0·3	8·0	− 7·7*	10·3	5·8	+4·5*
90	0	3·4	− 3·4	2·4	1·5	+0·9
155	0	0·5	− 0·5	0·8	0·6	+0·2
254	0	0·1	− 0·1	—	—	—

* Differences significant.

The results are shown in Table II, and are plotted as log. local lesions against time in Fig. 1. It will be seen that aeration greatly increased the rate of inactivation, and the exclusion of air by nitrogen retarded it, though not in so marked a degree. The reason for the somewhat irregular form of the curves has already been stated: a summation of the two sets of controls, however, gives the uppermost set of points, which can be fitted fairly well with the usual straight line form of inactivation curve. The linear tendency is, moreover, apparent in all the curves. The advantage of sacrificing uniformity over the total range to increased uniformity within each individual time comparison is evident when it comes to considering the significance of the higher values obtained for the nitrogenated series. Although considered singly only two time samples gave a significantly higher value for the nitrogenated inoculum, the plan of the experiment makes it legitimate to treat the successive comparisons as a set of replicates whose mean differences can be tested for significance by "Student's" method. The last five pairs of log. values were taken, since the first pair represented portions of the same inoculum before nitrogenation began. The mean difference thus determined (0·126 ± 0·034) was clearly significant, so that there is little doubt that the exclusion of oxygen delayed the inactivation of the virus.

CHANGES IN THE RATE OF INACTIVATION BROUGHT ABOUT BY OXIDISING AND REDUCING AGENTS.

The experiments just described suggest the susceptibility of spotted wilt virus to oxidation, but they also show that free oxygen in any quantity is unnecessary for its inactivation. In Fig. 1 the curve for the nitrogenated inoculum, after an initial rise above its control, thereafter runs almost parallel with it. There has thus been no great change in the rate of inactivation as a result of excluding all but traces of free oxygen. This does not rule out the possibility that inactivation is an oxidation reaction. On the one hand the nitrogen in this experiment may not have been entirely free from oxygen; or on the other hand the reaction may have been anaerobic, of the kind that involves the exchange of hydrogen atoms or electrons, for the juices containing the virus contained also the enzymes, proteins and other substances expressed from the plant.

The following experiments with oxidising and reducing agents show that definite changes in the inactivation rate of spotted wilt virus can be brought about, the most striking effect being the prolongation of the longevity of the virus from 3 hours to about 36 hours by the addition of sodium sulphite at a strength of 0·1 per cent. or of that order.

A list of the reagents[1] tested is as follows:

Oxidising agents:	Chloramine T. Hydrogen peroxide.
Reducing agents:	Sodium sulphite. Sodium nitrite. Ferrous sulphate. Tannic acid. Cysteine hydrochloride. Hydroquinone.

[1] These reagents were chosen from a rather limited range available, as substances which might possibly have an oxidising or reducing effect. It was realised that they might have other effects in the direction of precipitating colloids, altering hydrogen-ion concentration, etc., and, as stated later, the co-operation of a physical chemist has been obtained for further work taking account of oxidation-reduction potentials and other physico-chemical factors.

They were used at first at an arbitrary strength of 0·1 per cent., but other concentrations of some of the reagents were also tested. They were made up in solution at double the final concentration: so also was the virus, and equal quantities of the two were mixed. Samples were then taken at intervals and compared by the half-leaf method on tobacco leaves with a control virus from the same original extract, diluted only with distilled water.

Oxidising agents.

Chloramine T (chlorazene) at a strength of 0·1 per cent. of the virus suspension destroyed the virus so quickly that immediate inoculation gave no lesions at all.

Hydrogen peroxide at 0·01 per cent. final concentration caused no immediate diminution of virulence. At 0·05 per cent. the treated inoculum became inactive in somewhat less than 1 hour, whereas the untreated inoculum was still slightly active at 3 hours. At a strength of 0·1 per cent. inactivation was fairly rapid. In one experiment inoculations begun 1 min. after the addition of hydrogen peroxide gave for the untreated inoculum 101·6 lesions per half-leaf and for the treated inoculum 46·1 lesions. There were eight replicates and the mean difference was 55·5 ± 6·9.

Reducing agents.

Of the six reducing agents tried, four increased the rate of inactivation and two (sodium sulphite and sodium nitrite) decreased it.

Of the former, tannic acid and cysteine hydrochloride at a strength of 0·1 per cent. destroyed the activity of the virus almost instantaneously. Hydroquinine was tried at several strengths between 0·1 and 1 per cent., and in acid and in alkaline solution, but in all cases it destroyed the virus almost immediately. Ferrous sulphate at 0·1 per cent. caused a very rapid inactivation. In one experiment where inoculation was begun 3 min. after the ferrous sulphate was added to the inoculum the mean number of lesions per half-leaf (twelve replicates) was 7·4 as against 61·7 for the control.

Sodium sulphite is by far the most effective substance of those listed above for prolonging the activity of the virus. At a temperature of 20–22° C. the virus remained active for more than 36 hours in a 0·2 per cent. solution of the anhydrous salt, a control inoculum diluted at the same time with distilled water being completely inactivated in less than 7 hours.

Table III shows the effects of different concentrations of sodium sulphite on 1 : 30 inoculum at a temperature of 24° C. Each concentration was tested on a separate lot of plants so that the figures for equivalent time samples are not directly comparable: nevertheless as the test plants were not widely different they give some idea of the relative virulence of the different inocula. There is a marked optimum at 0·1 per cent. Another experiment was done at a temperature of 19° C. with inoculum from which air was excluded by nitrogen, and in which the concentrations of sodium sulphite ranged from 0·05 to 0·225 per cent. The greatest concentration prolonged the virulence of the inoculum most. No further attempts have yet been made to reduce the limits within which the optimum must lie.

Table III.

Effect of sodium sulphite in various concentrations on the longevity of spotted wilt virus. Mean numbers of local lesions produced on single leaves.

Mean inoc. time (min.)	Concentration of anhydrous sodium sulphite %				
	0	0·001	0·01	0·1	1·0
5	34	115	104	196	127
70	0·8	0·5	3	165	16
140	0	0	0·2	91	0
230	0	0	0	76	0
640	0	0	0	18	0

Fig. 2 shows the form of the ageing curve obtained when sodium sulphite is added to the virus, in this case at a final strength of 0·1 per cent. The upper curve, which shows ageing between zero time and 8 hours, is constructed with the accuracy obtainable from sixteen replicates for each time sample, whereas the lower curve, showing ageing of the same lot of virus up to 18 hours, is constructed from only six replicates for each time sample on a different series of plants.

Apart from the initial drop evident in the two curves, it is obvious that the usual linear relationship between the logarithm of the number of primary lesions and time holds here also. Had there been a control virus without sulphite in this experiment its inactivation curve plotted on the same graph would have been a straight line extending steeply down to the base line at about 4 hours. The effect of the added sulphite has been to change the slope of the inactivation curve but not to change its form.

Referring again to the initial drop observed in both curves of Fig. 2, in this case the inoculum was prepared by grinding leaves of diseased tomatoes under a 0·1 per cent. sodium sulphite solution. A similar preliminary fall was noticed in another experiment in which the inoculum was prepared in this way, but in all other experiments, in which the sulphite was added after extraction of the juice, no sign of such an initial drop was observed. The matter has not yet been further investigated.

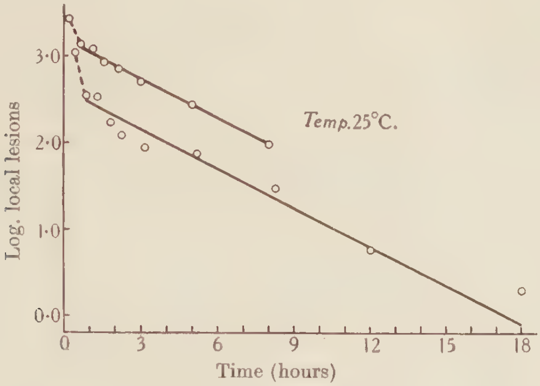

Fig. 2. Ageing curve for spotted wilt in the presence of 0·1 per cent. sodium sulphite. Each point on the upper curve represents the sum of the lesions on sixteen replicates. Each point on the lower curve represents the sum of the lesions on six replicates inoculated with the same sample of inoculum, but on a different set of plants.

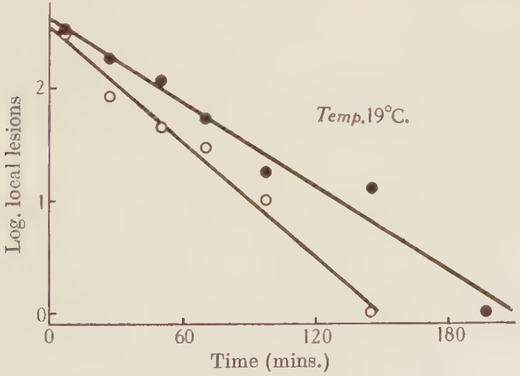

Fig. 3. The effect of adding sodium nitrite to spotted wilt inoculum. The upper curve represents ageing in the presence of 0·1 per cent. sodium nitrite, and the lower curve ageing of the control inoculum containing no nitrite.

Sodium nitrite was also found to increase the longevity of spotted wilt virus, although not nearly to the same extent as sodium sulphite in the concentrations tested. Again there was an optimum concentration in the neighbourhood of 0·1 per cent. (Table IV), and the inactivation curve was found to be of the same form. In the experiment illustrated in Fig. 3 where log. lesions is plotted against time, there were sixteen replicates for each time sample, but high temperatures in the glasshouse during the week before the experiment caused great variability in the test plants, and this is reflected in the scatter of the plotted points. Nevertheless the points fall about two diverging straight lines and when the regression of log. local lesions on time was calculated for both sets of data, the regression coefficients were found to be significantly different.

Table IV.

Effect of sodium nitrite on longevity of spotted wilt virus. Mean numbers of primary lesions on sixteen replicates.

Mean inoc. time (min.)	Concentration of sodium nitrite %					Mean of five treat-ments
	1	0·1	0·01	0·001	0	
8	—	126	116	98	114	113
50	46	77	42	33	43	48
109	24	28	3·6	2·5	6·4	13
146	12	22	4·3	1·6	4·1	8·8
249	0·8	1·8	0·5	0·4	0	0·7

REACTIVATION.

The work of Perdrau (1932) suggested that a watch should be kept for a possible increase of active virus particles soon after adding a reducing agent. Such an effect has, indeed, been observed at various stages of inactivation although not when inactivation was complete. After several preliminary experiments which gave definite indications of the increase, a satisfactory method of demonstrating it was found as follows:

Sixteen uniform tobacco leaves were chosen and a sample of inoculum prepared in water. Eight half-leaves were inoculated from the bulk sample, which was then divided, and to one-half was added 0·1 per cent. of solid sodium sulphite. Inoculation was then continued on halves of the remaining eight leaves with the untreated part of the inoculum. The test inoculum containing sulphite was then inoculated on the opposite halves of the sixteen leaves in the original order. The sulphite was thus added at the mean inoculation time; and assuming that ageing expressed as log. local lesions against time was linear in the control inoculum, the effect of differential ageing during the inoculation should not affect the comparison of test and control except slightly to the disadvantage of the test inoculum.

There were sixteen replicates of the comparison, and the mean difference between log. values for test and control was 0·148; the standard error was 0·0179, and the difference was therefore highly significant. The difference can only have been due to some form of activation or increase in virus particles, possibly by a reversal in the first stages of a chemical reaction causing inactivation.

CONCLUSION.

The evidence above does not yet decide the question whether the inactivation of the virus of tomato spotted wilt on standing is due to a reaction of the nature of an oxidation. Though the exclusion of air delayed inactivation only slightly, there remains the possibility that the nitrogen used was not absolutely freed from oxygen and that minute traces are sufficient to cause inactivation; or that inactivation is due to oxidation of an anaerobic type. The opposite effects of two such reducing agents as sodium sulphite and cysteine hydrochloride may have been due to differences in hydrogen-ion concentration, or to various unrelated properties of the introduced ions. In particular, nothing was known of the oxidation-reduction potentials actually induced in the inoculum by the added substances. Further work is now being carried out in co-operation with Mr R. J. Best, physical chemist at the Waite Institute, in which account is being taken of these factors.

SUMMARY.

Tomato spotted wilt inoculum stirred in the process of inoculation lost its virulence more rapidly than inoculum standing undisturbed.

The rate of inactivation of the virus was increased by bubbling air through the inoculum.

When all but traces of free oxygen were excluded by bubbling nitrogen through the inoculum loss of virulence was slightly delayed but the final rate of inactivation was practically unchanged.

The addition of two oxidising agents, chloramine T and hydrogen peroxide, increased the rate of inactivation.

Of six reducing agents tested, one, sodium sulphite, retarded inactivation very markedly. In one experiment at a temperature of 22° C. the virus remained active for more than 36 hours in a 0·2 per cent. solution of the anhydrous salt while control inoculum prepared at the same time with distilled water was completely inactivated in less than 5 hours.

Sodium nitrite retarded inactivation slightly.

The remaining four reducing agents—ferrous sulphate, tannic acid, hydroquinone and cysteine hydrochloride—hastened inactivation, but the oxidation-reduction potentials existing in the virus-containing systems were not known.

These results are briefly discussed in their bearing on the possibility that the normal rapid inactivation of spotted wilt virus which occurs in the standing juice is due to an oxidation reaction.

The authors wish to thank Mr R. J. Best and Dr A. H. K. Petrie for occasional advice in the course of this work.

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THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

V. THE MOVEMENT OF THE VIRUS AGENT IN TOBACCO AND TOMATO

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(With Plate X.)

In earlier papers various experiments were described which indicated that the virus agent is unable to enter unbroken cells and is unable to pass into or through dead tissue. This was interpreted to mean that the agent passes from cell to cell by the protoplasmic connections, and that protoplasmic continuity is necessary either (*a*) to carry the agent bodily from cell to cell, or (*b*) to transmit the toxin which gives rise to the virus symptoms.

It was shown that the virus of yellow mosaic of tomato moves freely about the plant, that it does not enter the water stream, and that when injected mechanically into the xylem vessels it is unable to leave them to enter the living cells. Some evidence was available also to show that the presence of phloem tissue proper is not necessary for the movement of the agent through the plant, and that movement can and does take place through living parenchymatous ground tissue. It was suggested that there was good reason for supposing that the virus agent travels from cell to cell along the protoplasmic strands, and that movement in the phloem takes place by virtue of the fact that the phloem is alive rather than by mass movement in which the virus is carried bodily along. These conclusions seemed to be borne out by the observations on the approximately equal rate of movement of virus both upwards and downwards in the plant. These observations had been made by Böning(3) and by the writer.

The work of Bennett(1), on the other hand, with raspberry mosaic in raspberries tended to show that this virus travelled more definitely in the phloem, and "ringing" experiments seemed to indicate that the extra-cambial tissues were the paths of translocation in this case. It is possible, however, that ringing may have the effect of destroying the living tissues of the xylem and medullary regions.

McCubbin and Smith (10) and Böning (3) have measured the average rate of movement of the virus, and their data confirm the view, put forward in an earlier paper of this series, that the movement of the virus in tomato is not very rapid, even having regard to the fact that the virus, after inoculation into a leaf, does not appear to leave that leaf for some 48–72 hours.

Holmes (9), on the other hand, has shown that the virus of tobacco mosaic in tobacco spreads slowly for some days following inoculation, and that it moves rapidly thereafter along the veins and stems. Samuel (11) goes so far as to say "in an area closely corresponding to where the virus has spread starch formation is inhibited so that the path followed by the virus shows up as a light coloured area (after iodine treatment) in the treated leaves. An intimate relation between the vascular tissue and the path of travel of the virus is made strikingly evident by this treatment." In Holmes's experiments, while virus multiplication was found to occur in the inoculated leaf, or portion of leaf, little increase of the virus was found in the leaves of the other side, or in the opposite part of the lamina of leaf of which only a portion had been inoculated. The distribution of the virus was very variable and, in some portions of the plant, large quantities of virus were found after only a few days, while in others no virus was recoverable until many days had elapsed.

It seems, therefore, clear that the movement of the virus in the host is less simple than had previously been supposed, and this paper deals with some data which have been obtained in experiments designed to throw further light on the subject.

THE MOVEMENT AND MULTIPLICATION OF THE VIRUS.

One point must be considered before proceeding to the experimental work. No one, apparently, has so far dealt with the problem of whether the virus is multiplied in given portions of the plant, *e.g.* at the site of inoculation, and is thereafter transported bodily along the conducting elements as would be an elaborated food substance, or whether the virus multiplies as it moves along from cell to cell.

A determination of this point would throw considerable light on the nature of the virus. Holmes (9) seems to be of the opinion that the virus of tobacco mosaic in tobacco moves with the food substances. The earlier papers of the present series tend to the view that the virus was travelling, in tomato at any rate, independently of the movement of food. This would be in concord with the view that multiplication of the virus is coincident with movement, and, as a consequence, movement and multiplication can take place only in the regions of actively metabolising cells. To establish definitely that multiplication and movement were coincident would be difficult—the technique of virus investigation is still very crude, but the data given below throw some light on the problem.

MATERIALS AND METHODS.

The virus agents used in this investigation were the aucuba or yellow mosaic of tomato and Johnson's No. 1 mosaic of tobacco. They were transmitted by juice inoculation in the hosts tomato—(*Lycopersicum esculentum*) var. "Kondine Red," and tobacco—*Nicotiana tabacum* var. "White Burley."

The juice was extracted in the manner described in the earlier papers and was usually filtered through fuller's earth before inoculation. This operation, which is easily carried out, apparently did not affect the virus agent and was satisfactory in removing from the macerated material the cell fragments and the chloroplasts which were present in it. Inoculations were made in every instance by rubbing leaves or parts of leaves with a cotton-wool swab soaked in the virus juice. This broke the epidermal hairs of the leaves without excessively damaging the mesophyll tissues. To assess the amount of virus present in the tissues, use was made of the plant *N. glutinosa*, which is particularly useful in this connection because the symptoms induced in the leaves by inoculation with either yellow mosaic of tomato or with tobacco mosaic are necrotic spots which are localised and which do not spread either over the rubbed leaf or from the rubbed leaf to others. Again, as has been shown by Holmes (8) and in the papers of this series, the number of spots which appear after inoculation is an index of the amount of virus present in any given inoculum.

THE APPEARANCE OF VIRUS IN TISSUES OF INOCULATED PLANTS.

In an earlier paper (6) it was noted that the symptoms of mosaic always appear on the developing leaves of the host plant, and it was suggested that this was due not primarily to the facts that the virus was not present elsewhere in the plant or induced chlorosis by the destruction of chloroplasts, but that the effect of the virus was to inhibit chlorophyll formation. This observation has been confirmed by Sheffield (12) working in this laboratory, who has studied the cytology of the diseased plants. From these observations it has become clear that the appearance of symptoms is not a necessary consequence of the presence of virus, and that virus may have passed through or may even be present in tissues without making obvious its presence. Again, the developing tissues in which symptoms appear, are also the most actively metabolising tissues and the most rapidly dividing, and the multiplication of virus seems to require rapid metabolism in the host cells. It does not follow, however, that the virus has not passed through other cells or even that the virus is not present in other cells in such small quantities as to make it difficult of demonstration. Experiments have shown that the virus can and does pass through tissues which do not show symptoms, and from which it is not possible to recover enough virus to demonstrate its presence.

In an earlier paper it was shown that the virus did travel through the tissues of a *Datura* plant and would cause symptoms in a tomato scion grafted on that plant, although the symptoms which appeared on the *Datura* stock were definitely localised and although no virus could be demonstrated in the tissues of the host plant, away from the place of inoculation. There is good evidence, therefore, for supposing that the absence of symptoms or even of the virus agent itself from any tissue is not necessarily proof that the virus has not at least passed through that tissue. Until the nature of the virus is more clearly comprehended, it is not possible to decide as to why some tissues should not retain the virus in them. Mention has been made of the fact that the chlorosis in mosaic diseases is due to an inhibition of the formation of chlorophyll by the tissues and that it, therefore, is most marked in the young and actively growing tissues. That the virus is also more abundant in the chlorotic leaves is easily demonstrated. Ten discs of tissue 5 mm. in diameter from the lower leaves of a tomato plant inoculated when it was fairly well grown were taken on April 28th. The plant had been inoculated on April 8th by rubbing the stem with virus juice. This ensured that the virus entered by the broken hairs without actually collecting on the surface of the leaves. The lower leaves of the plant were all free from symptoms of the disease although the upper leaves were chlorotic. The ten discs of tissue were macerated in 2 c.c. of water, and the whole was rubbed over the surface of some twelve leaves on two large plants of *Nicotiana glutinosa*. Similarly, ten discs of the yellow tissues of the upper leaves were taken and inoculated on the leaves of two other plants of *N. glutinosa*. The plants were kept in the glasshouse for a week, when it was found that the number of spots on the leaves inoculated with the yellow discs was much greater than that on the other leaves.

This result is probably not surprising, but that of another experiment of the same kind is more difficult of interpretation. There is evidence that leaves which were developing at the time of inoculation show, on maturity, symptoms of mosaic at the basal end, and are normally green at the apical end. The reason for this is discussed later in this paper. The leaves which have developed since infection, on the other hand, are also not uniformly yellow. On the young leaves there are areas of green tissue which is indistinguishable from the normal green tissues of healthy plants. These areas are found irregularly over the leaves and are not associated with absence of growth after infection or with any recognisable difference in the tissues. Discs of green and of yellow tissues were taken and macerated with water. The macerated material was inoculated on to leaves of *N. glutinosa* in the usual way.

After a week it was found that the leaves inoculated with the green discs had many fewer spots than those inoculated with the yellow discs. The proportion was of the order of 1 to 5. It is, therefore, clear that the quantity of the virus in the green areas is much reduced.

The green areas in the tomato leaves are not very extensive, and it is probable that small portions of yellow tissues were taken with the green discs. Support is lent to this view by the observations made on tobacco mosaic in tobacco. One of the characteristic symptoms of Johnson's No. 1 mosaic in tobacco is the presence of dark green areas slightly raised on the yellowish green lamina of the affected leaves. These green areas are similar in colour to the healthy tissues, and are in marked contrast with the chlorotic leaf tissues. They are often sufficiently extensive to allow of the disc being removed without accidentally including some yellow tissue. Ten discs of this and of the chlorotic tissue were taken and inoculated, after maceration, on to leaves of *N. glutinosa.* About twenty times as many necrotic lesions developed on the leaves inoculated with the yellow discs as developed on the leaves inoculated with the green discs. These results indicated that the multiplication of the virus is not uniform throughout the tissues of the host plant, and that not only do adult tissues not induce much multiplication but that there is also some factor, at present not clearly comprehended, which makes certain irregular areas of the developing tissues unsuitable for multiplication of the virus. A knowledge of the factors which prevent this multiplication would probably be of immense service in elucidating the problem of the nature of the virus. The observation is of interest also in connection with the transmission of the virus through the seed. If comparatively large numbers of the cells of ordinary vegetative tissue, scattered at random among the mesophyll cells, are unsuitable for the development of the virus, it is possible that similar conditions may obtain in the floral parts. It is not disputed that the fruits of the tomato, for instance, or of tobacco contain large quantities of virus but, even by those who hold that the embryos are affected, it has not been shown that more than a small proportion of the seeds do carry mosaic or streak. (See Berkeley and Madden (2).)

THE TRANSMISSION OF VIRUS THROUGH THE SEEDS OF TOMATO.

The question of the transmission of virus through the seeds of tomato has been for some time a vexed one. It has been variously held that the virus is, and that it is not, carried in the seed. Experiments of various kinds have been carried out, and some workers have shown that from 3–6 per cent. of the seeds of plants affected with streak or mosaic may carry the disease. Others have contended that the virus is never carried in the seed of the tomato. It is probable that experiments involving the inoculation of seedlings with crushed embryos from diseased plants may be discarded, because it is almost impossible to ensure that no trace of extra-embryonic tissue has been included in the inoculum. Since the fruits contain large quantities of virus the testa and integumentary tissues are necessarily infected. The experiments with growing seedlings are of more interest. The expression of symptoms of mosaic among seedlings is not different from that in older plants. Reference has before been made (5) to growing seedlings in cotton-wool impregnated with juice from aucuba diseased tomato leaves. When seeds are sown on such wool, or in soil watered with virus juice, germination is rapid, and the seedlings which develop are normal and are free from symptoms of disease. If, however, the rootlets or the cotyledons are damaged as they appear, symptoms appear on the young plants within a week and the chlorosis is found on all the leaves, including the first opened.

It is evident that there is no inherent cause for the non-development of symptoms in seedlings. If, therefore, symptoms do not appear at all or do appear only after the leaves have developed for some 2 or 3 weeks, there is reason for supposing that, in the first instance, the embryos did not contain virus, and in the second, there may have been secondary accidental infection. This does not preclude accidental infection taking place in the seedlings which show symptoms from the beginning—as in them infection may take place through damaged tissue rubbing on the testa. If this accidental infection did take place, since it would occur in the stage before the formation of the foliage leaf, the symptoms should appear on that and subsequent leaves.

In our glasshouses we raise some 8000 seedling tomatoes each year, and there has been no case of an indubitable seed infection in the last four years. This, of course, may be accounted for by the fact that seed had been taken from healthy plants. To avoid this possibility, at various times seed has been sown from plants with marked symptoms of aucuba mosaic or of streak. The seeds have been sown in flat boxes with some 300 seeds in each and have been kept in warm insect-proof houses. In no instance in some thousands of seedlings has any case of virus disease been observed. Having regard to the extreme ease with which these viruses travel about the plant, it would appear that the ovular tissue is for some reason free from virus, since the rapidly growing embryo would appear to furnish the ideal tissue for the multiplication of the virus if it were present. Further evidence for the multiplication of the virus in embryonic and rapidly metabolising tissue is given below.

Similar experiments have been carried out with tobacco seed from plants infected with different virus diseases, and again in this plant no evidence of seed-borne infection was observed.

In Table I is given a list of the viruses which have been examined with a view to discovering if they are seed transmitted.

Table I.

The seed transmission of the virus.

Host	Virus	No. of seedlings infected
Tobacco	Hyoscyamus III*	None
,,	Tobacco mosaic	,,
,,	Hyoscyamus III and "Green"	,,
Tomato	Aucuba mosaic	,,
,,	Tobacco mosaic	,,
,,	"Streak"	,,
,,	"Spotted wilt"	,,

* See M. A. Hamilton (7).

THE APPEARANCE OF VIRUS SYMPTOMS IN THE PLANTS.

Attention has been directed to the fact that virus symptoms appear in the growing tissues, and that the top portion of the plant is then the part in which the chlorosis is marked, if the plant were a fair size at the time of inoculation. A careful examination reveals the fact that among the leaves in the intermediate zone, that is, between those which are generally chlorotic and those in which no symptoms appear, there is a group of leaves—and this holds for both tomato and tobacco—in which the apical portion is apparently healthy and the basal portion chlorotic. The reason for this distribution of the symptoms becomes obvious when one remembers that the virus only inhibits chlorophyll formation and does not destroy preformed chloroplasts. The development of a leaf is most marked in the basal region, and that portion is the last to stop growing. As a consequence, therefore, it is possible that if the apical region of a leaf had at the time of infection developed its chloroplasts, this basal portion would only be developing, and could therefore be liable to attack as a consequence of the virus infection. The development of a leaf is easily demonstrated by marking the lamina of a developing leaf with squares 1 cm. × 1 cm. This was done for a developing tobacco leaf, and after a week a photograph of it was taken. It will be seen from the photograph in Plate X, fig. 1, that the marking was not geometrically accurate—the nature of the leaf of a tobacco does not allow of this—but that it is clear that the squares are much larger at the basal end than at the apical. At the same time, the Indian-ink lines are much less faint at the apical end of the leaf than at the basal. The growth, therefore, has been approximately twice as great at the basal end. It is, consequently, not surprising that the symptoms of virus disease tend to occur on that part of those leaves which were in the course of development at the time of inoculation.

The non-appearance of symptoms must not be taken as evidence of the absence of virus, but it does appear from the results which have been set out, that the virus increases very much more in tissues which are developing and which will, incidentally, show marked symptoms than in those tissues which have already developed.

THE MOVEMENT OF THE VIRUS FROM THE INFECTED LEAF.

Holmes has shown that the virus of tobacco mosaic moves in a peculiar way from the leaf where the inoculation was made. He attributes this to the fact that the virus travels with the food material, *i.e.* presumably in the phloem. The experiments now to be described were designed to examine this hypothesis. The first experiment was similar to those of Holmes. A large plant of tobacco some 18 in. high was selected, and inoculation was made by rubbing the inoculum on a small portion at the apical end of a mature leaf half-way up the plant. After 7 days the leaves were removed severally, and were crushed in a mortar with twice their own weight of water (the usual procedure for obtaining an inoculum). Each of the leaves was numbered. The side of the lamina on which the inoculation was made was numbered 1*a*, the opposite side 1*b*. The distance from the place of inoculation to the base of the leaf was 15 cm. The next higher leaf on the stem was numbered 2, the next 3, then 4, then 5, and the apical region of the stem, together with its leaves 6. The leaf next below the inoculated one was 2^1, the next lower 3^1, and the next again 4^1. Each of these inocula was rubbed on the leaves of plants of *N. glutinosa*, which develops, with this virus, necrotic lesions. At the time of sampling the upper portion of the tobacco plant showed symptoms of mosaic. The results obtained on the fifth day after rubbing the *N. glutinosa* leaves are detailed in Table II.

Table II.

Number of lesions on leaves of N. glutinosa *inoculated from the leaves of tobacco.*

Leaf 6 (including top of plant) ...	Many lesions
Leaf 5	Many lesions
Leaf 4	Very many lesions
Leaf 3	Many lesions
Leaf 2	No lesions
Petiole of infected leaf	Some lesions
Leaf 1*a*	Few lesions
Leaf 2^1	Very few lesions
Leaf 3^1	No lesions
Leaf 4^1	No lesions

This is not quite in accord with the data of Holmes, but the difference is, I think, attributable to the fact that the leaves on the opposite side of the plant in this experiment, viz. leaves 3 and 4, were not quite fully developed at the time of infection. In other words, the amount of virus present in a leaf at any given time depends not so much on whether the virus has reached that leaf as on whether, having reached it, multiplication has taken place. Leaf 2 illustrates this point; there is little reason inherently why the virus should not have reached leaf 2 when it has reached leaf 3, and no matter what the mechanism or path of movement may be, the virus must have passed within a maximum of a centimetre of leaf 2 to get to leaves 3 and 4, etc.

To discover if the absence of the virus from leaf 2 immediately above the infected one were due to the non-multiplication of the virus in the mature tissues of that leaf, a second experiment was set up. A plant, rather larger than the last was selected, and all the leaves with the exception of one half-way up the stem were removed. An inoculation was made on a small area of this leaf on one side of the lamina at the apical end. This area was 30 cm. from the base of the leaf. On the ninth day symptoms appeared on the leaves of the axillary buds which had developed in the interval. These axillary shoots corresponded to all the leaves on the plant, both above and below the treated petiole, and this would suggest that the presence of the embryonic tissues did induce the appearance of the virus in quantity in those regions where, when the adult leaves were present, it was in so small quantity as not to be demonstrable. Further evidence in support of this view is afforded by this additional observation.

The leaf on which the inoculation was made was divided into three parts: (1) the half-lamina on the same side as the inoculation, less the inoculated portion, (2) the other half-lamina, and (3) the mid-rib and the portion of the lamina immediately round it. The three portions were each macerated in water, and the inoculum rubbed on the leaves of plants of *N. glutinosa*. After a week these leaves were examined, when it was found that the lesions had developed only on those which had been inoculated with the material from the mid-rib.

The virus had multiplied in the region of the original inoculation where local symptoms appeared, and also in the mid-rib of the leaf. Multiplication had apparently not taken place in the other portions of the lamina. It is probable that the breaking of the cells set up some increase in the metabolism of the injured tissue, as one would expect, and that this accounts for the increase round the point of inoculation. Similarly there is probably more activity round the vascular tissue than in normal mesophyll cells and this, in turn, may account for the multiplication in the mid-rib. Alternatively, the movement down the mid-rib may be interpreted as indicating that the virus is being carried with the food material in the vascular tissue. This hypothesis is examined in the next section.

THE POSSIBILITY OF MOVEMENT OF THE VIRUS WITH THE FOOD MATERIAL.

It might well be argued that the fact that the older leaves do not develop virus might be due to the passage of all the food material downwards from the lamina into the main stem. Since the virus does not travel in the xylem (4) but in the living tissue, the phloem might be the path by which movement took place, if that movement were due to the removal of the virus, passively, from place to place, with the food material. To clear up this point the following experiment was carried out. Some large tobacco plants were put in a warm, dark chamber for 2 days so as to allow of the removal of the reserve carbohydrates from the mesophyll tissues. The next day all the leaves but one, half-way up the stem, were removed. An inoculation was made with tobacco mosaic on an area of the remaining leaf in the same manner as before (see above), except that immediately after inoculation the leaf was completely darkened by being enclosed in a black cloth envelope which excluded the light. The effect of this treatment should be to reverse the normal movement of the food materials from this leaf. Since it is fully grown, it is presumable that the foodstuffs elaborated in it are sent down the petiole and are sent thereafter to the embryonic tissues. Under this treatment, however, the other tissues of the plant should actually be elaborating more carbohydrate than are those of the inoculated leaf, and, consequently, the movement of the food material should be towards and not away from the treated leaf.

After an interval of 6 days symptoms appeared on the axillary shoots which had developed as a consequence of the mutilation. The leaves of all the axillary shoots developed symptoms, whether or not the inoculated leaf had been darkened. These tissues in which the symptoms appeared correspond exactly to the leaves of the plants in the earlier experiments, and this observation confirms the idea that a mature leaf may not induce the multiplication of the virus, whereas a rapidly growing one under the same conditions would. At the same time, the fact that darkening the inoculated leaf has no apparent effect on the infection of the plant, suggests that the amount of the virus is not dependent on that of the food supply.

This second point is confirmed by another experiment. Some large tobacco plants about a foot high were put in complete darkness for 48 hours. Thereafter they were inoculated with tobacco mosaic and returned to the light in a glasshouse. One set was inoculated on the uppermost leaf of inoculable size, the second set was inoculated on the third foliage leaf from the base and the third set was inoculated on the same leaf (the third from the base), this leaf being subsequently covered with a black cover which excluded the light. On the seventh day after inoculation all the plants showed symptoms of tobacco mosaic, there being not the slightest evidence that the virus in any one set had reached the apical region before that in the others.

A similar experiment was set up with tomatoes and aucuba mosaic. The tomato plants were about 9 in. high at the beginning of the experiment. They were kept for 48 hours in complete darkness. At the end of that period a small portion of the tissue of the stem above the axil of the second foliage leaf proper was removed. This had the effect of cutting off the direct vascular connection between this leaf and the upper portion of the plant. The appearance of the treated stem is indicated in Plate X, fig. 2. As a consequence of this it was expected that the axillary bud below the cut portion would grow immediately—as indeed it did.

The plants were then divided into three groups. The plants of the first group were inoculated with aucuba mosaic on the topmost leaf, which was sufficiently developed to allow of inoculation. The plants of the second group were inoculated on the distal pinna of the leaf below the notch in the stem. This was a fully mature leaf, and it was not expected that symptoms would appear on that leaf. The plants of the third group were also inoculated on the second leaf above the cotyledons, and this leaf after inoculation was inserted into a black hood which excluded the light. The expectation was that the carbohydrates of this leaf had been completely used up, and that such carbohydrates as it obtained would be obtained from the rest of the plant. Here again, therefore, if there were a movement of food materials, it would be *into* the leaf and not out of it. Five days after inoculation all the plants, which were kept in a warm glasshouse, showed symptoms of aucuba mosaic on the top leaves. The axillary bud below the notch in the stem had developed in the interval, and its leaves also showed signs of mosaic in all cases. The symptoms appeared just as quickly on the plants with the darkened leaves as on the others. It appeared, therefore, that the virus had travelled up the plant from the lower rubbed leaves, and down the other plants from the upper rubbed leaves of the first and second groups respectively at approximately the same rate and that, despite the removal of the direct vascular connection of the lower axillary bud with the upper portion of the plant. At the same time the virus had multiplied in, and had moved out of, the darkened leaf and had moved about the plant apparently at the same rate as in the others, despite the fact that there could have been little, if any, movement of food material from that leaf. In all the plants, the lowermost leaves, including that in the axil of which the shoot grew out, showed no symptoms of virus, being at the time of infection fully mature. These experiments were repeated, use being made of the axillary shoots corresponding to the treated leaves. Similar results were obtained.

Discussion.

Two main lines of work have been dealt with in this paper, viz. (1) the non-transmission of virus through the seed of tomato and tobacco, and (2) the movement of the virus as compared with that of the food materials in the plant.

The experiments with seedling inoculation show that there is no inherent reason why a young plant should not develop symptoms of virus. It has been found that, provided the tissues be broken, no matter how early infection be made, with either aucuba mosaic or "streak" virus, symptoms will appear on the first foliage leaf when it develops. It is, therefore, probable that virus which was in the embryo should induce symptoms in all the foliage leaves of the seedlings. If infection should take place by rupture of the hairs or tissues of the embryo and entry of the virus adhering to the integumentary tissue, again, symptoms would appear in the first-formed foliage leaf. In no instance, in the hundreds of seedlings grown from seed taken from infected plants, has a single seedling been found in which, without actual inoculation, virus symptoms have developed. Inoculation in the usual way from those seedlings into other healthy seedlings has in no instance given evidence of the presence of the virus in a latent form.

The examination of the embryo in the early stages shows that growth of the embryonic tissue is very rapid, as also is the increase in size of the embryo. The difference in growth between the embryonic tissue and the normal ovular tissue would have the effect of breaking the protoplasmic connection between the embryo and the placental tissue so that the virus would normally have no opportunity of entering the embryo after development had begun. This would account for the absence of secondary infection of the embryo, but does not take account of the possibility of infection of the megaspore mother-cell at a much earlier stage. Reference has been made to the presence in the tissues of groups of cell which for some reason are apparently more resistant to the virus, or prove unsuitable for the multiplication of the virus within them. It may be that potential megaspore mother-cells are of this type. That the reproduction tissue proper may be unsuitable for the multiplication of virus is further borne out by the fact that I have been unable, so far, to demonstrate the transmission of the virus through the pollen. The amount of pollen which is available at any time from the flowers of virus diseased plants is not very large and the experiment, therefore, is not a highly critical one—the results being tentative rather than conclusive.

The experiment is further complicated by the fact that it is difficult to ensure that no pieces of tissue will be shaken off with the pollen grains and the occasional spots which do appear on the leaves of *N. glutinosa* after treatment are believed to be due to accidental infection, either from the surface of the pollen grains or from hairs, etc., which had broken off.

The procedure consisted of shaking the open flowers in a small glass dish into which the ripe pollen fell. This was subsequently macerated in water. As can be seen, there is considerable danger of other tissues being collected with the pollen, and so odd spots were discounted, it being felt that the thousands of pollen grains would, had they been infected, have supplied a great deal more virus than that found.

The results of the experiments on the movement of the virus in the plants indicate that the virus moves independently of the food materials. It would appear, therefore, that the virus is not carried mechanically from some source in which it has been elaborated, but rather that it moves outward in all directions from the place of infection, along the protoplasmic strands and increasing in amount as it passes from cell to cell. The mechanism by which this increase takes place is not yet understood.

SUMMARY.

Experiments on the movement of the virus of aucuba or yellow mosaic of the tomato in the host plant are described. It has been found that the presence of the virus in the tissues is not always associated with symptoms, and that the symptoms appear in those tissues which have developed after infection. It was also found that the distribution of the virus throughout the plant was not uniform. In the chlorotic tissues the virus content was higher than in the neighbouring green areas.

A large series of experiments has been carried out on the transmission of six different viruses in the seed of tomato or tobacco. In no instance was there any evidence of transmission, and it is suggested that the chance of seed transmission of these viruses is very slight.

The movement of the virus from an infected leaf and the possibility of its being carried with the food material were examined, and results are given which show that the virus can move independently of the food materials and that, under certain conditions, the virus apparently moves in the direction opposite to that of the metabolites.

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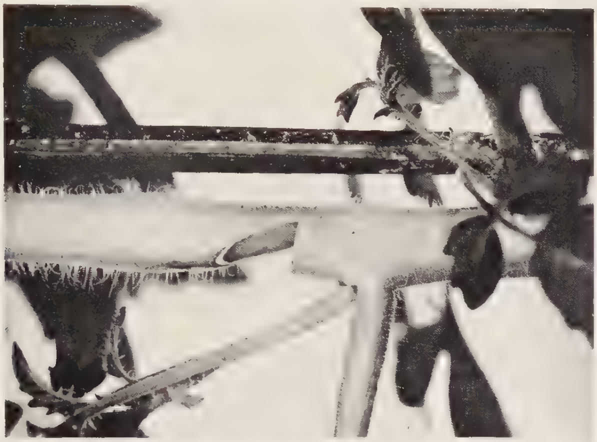

Fig. 2.

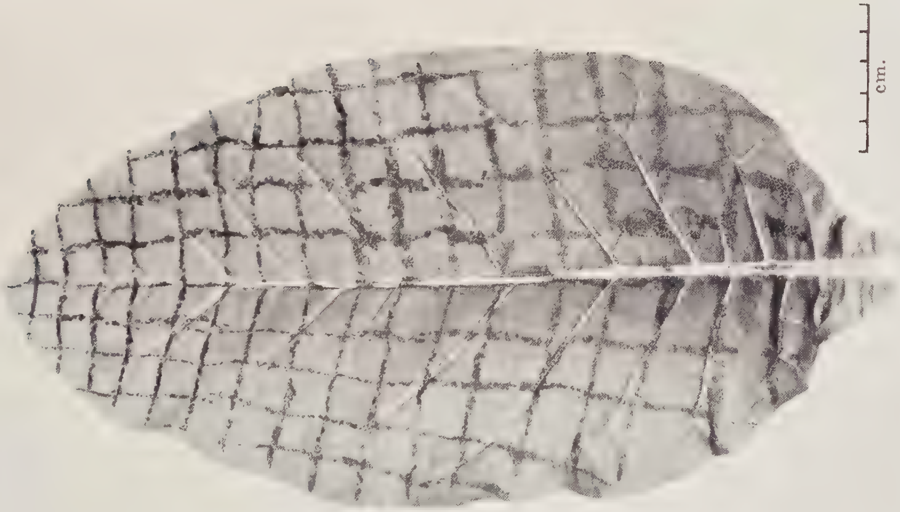

Fig. 1.

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EXPLANATION OF PLATE X.

Fig. 1. A leaf of tobacco which had been marked with 1 cm. squares a week previously.

Fig. 2. A wedge-shaped portion removed from the internode of a tomato plant to isolate a leaf and its axillary bud from the upper portion of the stem.

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THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

VI. SOME EFFECTS OF MOSAIC ON THE METABOLISM OF THE TOMATO

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(With Plates XI and XII and 7 Text-figures.)

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INTRODUCTION.

ALTHOUGH the effect of virus diseases on the metabolism of the host plant must obviously be considerable, the physiological study of the diseased plants has, generally, been neglected in the past in favour of the examination of their cytology and symptomatology. Various workers have, from time to time, reported on the alteration of the metabolism of plants as a consequence of virus diseases, but the analyses which have been made have been largely concerned with the chemical aspects, and little work has been carried out on the more strictly physiological side.

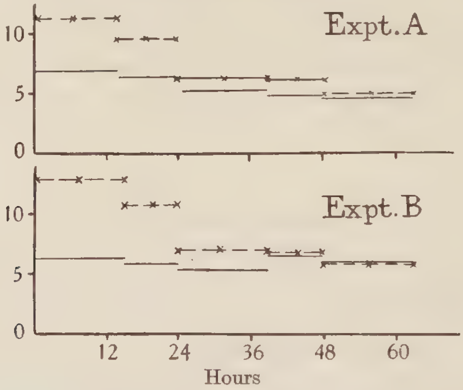
The two main processes which would most likely be affected by virus diseases are photosynthesis and respiration. That virus diseases have a definite effect in many instances on the growth of the host is evident, but the analysis of growth is difficult and not sufficiently worked out to make available much of value from its study in this connection.

Photosynthesis, which so often has been used in the past as an index of metabolism, is in these diseases of less interest than respiration because of the obvious reduction in chlorophyll content which is usually characteristic of the disease. The reduction of the amount of chlorophyll would necessitate a complicated examination of the efficiency of the photosynthetic activity if this were to be used as a criterion of metabolism. In respiration, however, there is a system, the action of which is continuous, and on which external conditions exert an influence which is fairly clearly comprehended.

PREVIOUS WORK.

There are in the literature various papers dealing with the chemical metabolism of virus-diseased plants. Woods (17) found that the oxidase content of diseased tissue was higher than that of normal. Some of his conclusions, based on the results of his work, have been challenged by Allard (1), and it is probable that he has, as Freiberg (11) suggests, mistaken the effect for the cause. Bunzel (3) found that the curly-top disease in sugar beet had the effect of reducing the sugar content of the leaves, while the nitrogen content remained unchanged. In the roots, however, the total nitrogen was increased and the sugar content decreased. Jodidi (12, 13) and others worked on the alteration in the nitrogen content of spinach and cabbage caused by virus disease and found a decrease of nitrogen in the diseased plants. True *et al.* (15) found an accumulation of carbohydrate in diseased spinach. Freiberg (11) found differences in the carbohydrate content in the light and dark green areas in tobacco with mosaic. Campbell (5) found that the leaf-roll disease of potato induced the accumulation of carbohydrates while it had no effect on the nitrogen content. Many workers have dealt with the interesting problem of the accumulation of starch in the leaves of potatoes with leaf roll and that of the translocation of the sugars through the necrosed stems.

Few workers have attempted to assess the effect of virus disease on the fundamental metabolism of the diseased plants. Thung (14), working at Wageningen, studied the effect of leaf roll on various aspects of the metabolism of the potato. One of the aspects on which he touched was the respiration as measured by the CO_2 output. He found that the CO_2 output of the diseased tissue was higher per gram dry weight or wet weight per hour than was that of the normal. Taking into consideration the increased starch content of the diseased leaves, he concludes that the enzymatic processes are not influenced by the virus. An examination of the data which Thung furnishes on the liberation of CO_2 leads one to the view that his results are very inconclusive. The two curves in Text-fig. 1 of the present paper show the results of the two experiments which most nearly correspond to those described in the later portion of this paper, the data being plotted as is done later for that of the tomato experiments. It can be seen that the amount of CO_2 produced by the diseased tissue is not consistently more than that produced by the healthy, and that it would, in these experiments, be quite competent to argue that, had the experiment continued without injection of the leaves by water, the tendency would have been for the output of CO_2 of both sets of leaves to have reached similar values. From the values obtained it is questionably desirable to attempt to deduce any conclusions. As can be seen from the figure, almost any hypothesis might be advanced with more or less conviction and with equal justification.



Text-figure 1. Graphs showing the CO_2 output of detached leaves of healthy (solid line) and leaf-roll potato plants (broken line). (From Thung.)

Dunlap (6, 7) has more recently in a series of papers discussed the carbohydrate content of virus-diseased plants. He has found that as regards the reaction of the host, two main groups of virus diseases of the chlorotic type appear to exist. In the mosaics proper the effect of the disease is to increase the total nitrogen content and to decrease the carbohydrate content of the affected plants. In the yellows diseases, on the other hand, the converse holds, *i.e.* the nitrogen is decreased and the carbohydrate increased. The C/N ratio of mosaic leaves was therefore reduced and that of yellows increased. Respiration was found to be increased in young tissues affected with virus diseases and to be decreased in older tissues, as compared with the healthy controls. The method Dunlap used to determine the CO_2 output is not above suspicion, and it would appear that, to some extent, he was measuring not respiration in air alone but the CO_2 output of leaves in an atmosphere of less than normal oxygen content.

Brewer *et al.* (2), working on the effect of mosaic of tomato, found that a reduced carbohydrate content and a normal nitrogen content was characteristic, this giving a lower C/N ratio than the healthy. Woods has suggested that tomato mosaic causes an accumulation of starch in the leaves.

Elmer (9) has shown that the effect of mosaic disease in tomato is greatly to alter the composition of the chlorophyll, and Eckerson (8) has demonstrated cytologically the disintegration of the chloroplasts in tomato mosaic, though her observations mainly concerned themselves with a motile organism, the existence of which as the virus agent has not been established.

MATERIALS AND METHODS.

For the experiments here recorded the virus disease aucuba or yellow mosaic of tomato was studied in the host *Lycopersicum esculentum* var. Kondine Red, or in tobacco *Nicotiana Tabacum* var. White Burley. Where this arrangement has been departed from specific mention is made: otherwise the procedure was the same in all cases. Leaf tissue of infected plants of tomato was macerated with water in a mortar in the proportion of 1 gm. of tissue, other than petioles and midrib, to 2 c.c. of distilled water. This material was allowed to stand on one side, usually overnight, in the laboratory. It was then filtered through muslin and all the larger masses of tissue removed. Thereafter it was passed through fluted filter paper impregnated with fuller's earth. This had the effect of removing all the suspended chloroplast material and cell débris and did not apparently reduce the virulence of the agent. The resulting material was dark brown in colour and quite clear. This was used as the source of inoculum. Inoculations were made by rubbing the leaves of experimental plants with a piece of cotton-wool soaked in this liquid. The rubbing was done in such a way as to break as many hairs as possible without extensively damaging the mesophyll cells of the leaves. As a consequence of this treatment no local lesions appeared on the rubbed leaves in the tomato, but systemic infections appeared after an appropriate incubation period.

THE EFFECT OF THE DISEASE ON THE APPEARANCE OF THE HOST PLANT.

The most evident symptom and the one which has characterised the disease is the appearance on the tomato plant of yellow areas. These develop four or five days after inoculation in summer time and after a rather longer period in dull weather. As the leaves develop, the chlorotic areas do not increase in size so rapidly as the green tissues, and the adult leaves are crinkled and distorted. The affected leaves are always smaller in size than the normal, and in summer when the growth of the controls is very rapid the difference in size is very pronounced. It is abundantly clear from macroscopical observation that the effect of this virus on the tomato plant is to inhibit the formation of chloroplasts to a greater or less extent. It has been regularly noticed that in our cultures some plants were much more affected by the disease than others. This led to a detailed examination of the effect of the age of the plant on the response to infection. Mention has been made of the fact that the intensity of symptoms is dependent on the light conditions, and the general conclusion may be drawn that under bright light conditions, especially in summer, the symptoms are much more clearly marked and the chlorosis is more pronounced. At the same time, under similar conditions it has been found that cultures of plants inoculated as seedlings show very much more extensive distortion and chlorosis than those inoculated at a later stage of development. The reason appears to be, as has been pointed out in earlier papers of this series (4), that the virus induces symptoms only in those leaves which develop subsequently to inoculation, and that all leaves which are fully developed at the time of inoculation do not develop symptoms. The leaves which were developing at the time of infection are in an intermediate condition, and the symptoms occurring on them are variable. In any plant, therefore, which is infected at a fairly late stage of development there are three regions. In the upper part the leaves have all well-developed symptoms of mosaic, in the middle part the symptoms are less evenly distributed, often only a few yellow flecks being present, and in the lower part the leaves are normal in appearance. The non-appearance of symptoms is not indicative of absence of the virus agent from the tissues, and the virus can be extracted from all parts of an affected tomato plant after an appropriate interval of time.

The fact that the virus has no effect on the preformed chloroplasts and that the proportion of the plant deformed by the agent depends on the stage of development at infection has important effects on the metabolism of the host. The amount of reduction in the photosynthetic activity of the plant clearly depends at least in part on the reduction in the chlorophyll content. Since, therefore, the number of chloroplasts depends more or less on the leaf area of the plant, those plants which have been inoculated as seedlings will have the most reduced photosynthesis. The consequence is that the plants which have been inoculated as seedlings have no normal leaves whatsoever, and as a result of the initial reduction of chlorophyll content are much more markedly starved as compared with the controls than are those which, having been inoculated later, have a number of leaves on the lower part of the plant which by normal photosynthesis generally increase the size of the plants. Plate XI is a photograph of three plants all of the same age. At the time the photograph was taken they were three months old. That on the extreme left is the uninoculated control. The middle plant was inoculated with aucuba mosaic when it was in the fifth normal leaf condition, and that on the right was inoculated as soon as the foliage leaves were old enough to be inoculated with the virus. It can be seen that the lower leaves of the middle plant are normal in appearance and size, and that the symptoms are confined in the main to the upper leaves which are very much more reduced in size and are distorted and chlorotic. The leaves of the plant on the right are all very much reduced in size and are distorted and chlorotic. Clearly the fact that the middle plant is not so reduced in size as is the right-hand one is attributable to the fact that the lower leaves on the former were able to carry on normal photosynthesis and to produce carbohydrates which to some extent supplemented the reduced supply from the chlorotic leaves at the tip. The leaves of the right-hand plant having been reduced and chlorotic from the beginning had no normal supply of carbohydrates on which to draw and are therefore always reduced in size and the plant starved. This consideration is of importance in the study of the respiration of the healthy and diseased tissue and is more fully dealt with in a subsequent section.

THE STAGE OF DEVELOPMENT OF VIRUS-DISEASED PLANTS.

Despite the fact that the size of the plant is much affected by the disease and the metabolism is completely disturbed, the stage of development is unaffected by the virus. A study of the three plants in Plate XI, reveals the fact that despite the considerable disparity in size each of the plants is at the same stage of development. In each case the flowers of the first truss are just on the point of opening. There are the same number of leaves on each of the three although the leaves on the plant infected as a seedling are much smaller in size than those of the other two plants, and the upper leaves of the plant infected later are reduced in size compared with those of the control. This point is even more clearly seen in the two plants in Plate XII. The plant on the left had been inoculated with aucuba mosaic as a seedling, that on the right is the control of the same age. The appearance of the plants is very different, yet one is merely a reduced replica of the other. They have the same number of leaves, and the first flower truss on each can be seen projecting from the stem just above the branch which has developed in each from similar axillary buds.

THE EFFECT OF THE DISEASE ON THE DRY-MATTER CONTENT OF THE TISSUES.

The effect of the disease on the appearance of the plant has been discussed, and this section is devoted to a consideration of the effect of the virus on the dry-matter content of the plant. Some typical results are detailed below. The leaves of the two plants shown in Plate XII, were removed and weighed. The weight of the leaves was 88·0 gm. and 20·1 gm. for the control plant and the diseased respectively. The leaf tissues therefore of the diseased plant were very much smaller in amount than that of the control. This disparity was further borne out in the dry-matter contents. This was obtained by drying the leaves at 90° C. until such times as consecutive weighings varied by only 0·01 gm. The percentage of dry matter in the control was 12·9 per cent. and in the diseased plant was 7·8 per cent. Both plants had been grown under comparable conditions, and both plants were kept equally watered so that no temporary disturbance in turgidity should occur.

This difference is of the order expected in these experiments, and the general conclusion to be drawn from a considerable number of observations is that, in plants on which the inoculations have been made at an early stage and of which the whole period of development has been characterised by the reduction of chlorophyll and the subsequent general starvation of the plant, the dry-matter content is of the order of 7–8 per cent. In plants in which inoculations took place at a later date and which, therefore, before infection had developed normal photosynthesis, the dry-matter content was of the order of 10–12 per cent. In the healthy controls kept under similar conditions and grown in soil as nearly as possible equally moist the dry-matter content of the leaves was of the order of 14–15 per cent. The reduction in dry-matter content, expressed as a percentage, is therefore in the case of seedling inoculated plants as high as 50 per cent. and is very high in the chlorotic leaves of plants infected at a later stage in their development—about 30 per cent.

The same differences are to be found in the tops. The upper parts of the plants were removed, weighed and dried as were the leaves, and distinction could be drawn between the three groups in the same way as between the leaves of the plants. This difference is of interest in connection with the data on respiration.

THE EFFECT OF THE DISEASE ON THE CARBOHYDRATE CONTENT OF THE TISSUES.

The effect of the virus of yellow mosaic is to decrease the chlorophyll content of the tissues. As a consequence, the carbohydrate content will probably also be reduced. A complete survey of the alteration in the carbohydrates formed has not yet been made, but numerous estimations have been made of the total carbohydrate content of healthy and diseased tomato tissue. The analyses have been carried out by me and by two of my colleagues, Drs Norman and Jenkins, of the Department of Fermentation. Actually three methods were used, viz. a modified Fehling's method, the Hagedorn-Jensen method, and the Schaffer-Hartman method. The samples were collected usually about midday and were extracted for 1–2 hours with boiling 1 per cent. HCl in a sand bath. Thereafter the tissue was filtered off through muslin and washed with boiling water. After the filtrate had cooled it was made neutral to phenolphthalein with NaOH. The protein was precipitated with basic lead acetate and the excess lead removed with di-sodium hydrogen phosphate. The volume was then made up to 250 c.c. and samples taken for analysis.

As would be expected, the different samples varied very much from time to time, but there was consistently more carbohydrate in the normal than in the diseased tissue taken at the same time. The tops of plants inoculated as seedlings always contained least total carbohydrate, the tops of normal plants most, and the tops of plants inoculated after four or more foliage leaves had formed was intermediate in position. Table I shows the kind of differences found.

Table I.

The total carbohydrate content of tops of tomato plants.

Normal plant	Plant inoculated at 5th leaf stage	Plant inoculated as seedling
1·6 %	1·3 %	1·1 %

THE EFFECT OF THE DISEASE ON THE NITROGEN CONTENT OF THE TISSUES.

The effect of the virus on the nitrogen content of the plants is less marked than on the carbohydrate content. The total nitrogen was estimated by the Kjeldahl method. The results were by no means consistent—sometimes the healthy tissue had a slightly higher nitrogen content than the virus tissue and sometimes the reverse held. When the virus tissue contained the larger amount of nitrogen, the difference could usually be attributed to the lower carbohydrate content, and it appeared that the effect of the disease was not to affect the nitrogen content of the plants.

THE EFFECT OF VIRUS DISEASE ON THE RESPIRATORY MECHANISM.

Reference has already been made to the work of Thung and Dunlap on the respiration of tissues affected with virus disease. Thung worked on the diseased leaves of potatoes with leaf-roll, as also did Esmarch (10). Thung found that the CO_2 output of virus leaves was slightly higher than that of normal, and concluded, from this observation, that, since the starch content of the leaves is much increased in this disease and the respiration is not disproportionately increased, the enzyme system must be of the same order in both healthy and diseased. In other words, that the enzymes were not greatly affected by the disease. As has been noted, there are some criticisms of Thung's methods which suggest themselves, especially as regards the validity of the deductions from the rather tenuous evidence he has available.

Whitehead (16) has reported, shortly, on the effect of leaf-roll on the respiration of potatoes, and has shown that the effect varies with the stage of development of the tubers. In general, however, the disease has the effect of increasing the CO_2 output of the tubers in air. "Anaerobic respiration," he says, "is unaffected by the virus—the tuber whether diseased or not, producing 70–80 per cent. of the CO_2 evolved under aerobic conditions."

Dunlap's work has also been discussed above, and it has been suggested that the method he employed was not wholly satisfactory. Not only is it uncertain that the caustic soda would absorb the CO_2 which is liberated, but the period over which the experiment was carried out is probably too short to make the results conclusive. At the same time, short as the period was it was probably long enough to induce anaerobic respiration, so that various unconsidered factors may have entered into the experiment.

Dunlap's general conclusions are as follows: "mature diseased leaves produce less CO_2 than similar healthy leaves, in the case of both the mosaic and yellow types of disease—the respiration rates of young leaves were found to be somewhat increased by the diseased condition—there seems to be no consistent evidence that variations in the respiratory rate due to the diseased conditions are brought about by variations in the carbohydrates or nitrogen controls of the leaves examined." In general, the findings of the various workers who have measured the respiration of diseased tissues have been conflicting, and no very clear-cut data have been available.

METHODS AND MATERIALS.

In the experiments which are outlined below, the material used in the respiration chamber was healthy and diseased tissue of the tomato (*Lycopersicum esculentum*) variety Kondine Red. These plants were grown in the glasshouses here, and the diseased plants had been infected with aucuba or yellow mosaic of the tomato, either as seedlings or at a later stage. The inoculation was made by rubbing a few of the lower leaves with a cotton-wool pad, soaked in macerated tissue from diseased plants. The symptoms of this disease are very well marked in summer and consist of a bright yellow mosaic with some leaf distortion. In the later experiments, either leaves or the whole top of plants were used, and certain precautions were taken to note the time of infection of the host plant. The reason for this is obvious from the data set out below. A known weight of leaves, or of apical shoot and leaves ("top"), was put into a glass container. Two containers were used, one for the diseased material, the other for the healthy, and they were put together into a water bath which was kept at a temperature of 25° C. A stream of CO_2-free air, brought from outside the laboratory to avoid contamination with fumes, was led through each of the chambers. Each stream was then carried through a series of Pettenkofer tubes so arranged in the apparatus devised by Blackman that three hourly samples of the CO_2

liberated were collected in baryta in each of the tubes. The CO_2 was collected in $M/15$ $Ba(OH)_2$ which was back-titrated with HCl to measure the amount of free alkali. A simple calculation gave the CO_2 equivalent to the acid required to neutralise the free baryta—phenolphthalein being used as the indicator.

In the first experiments, the material, either leaves or tops, was chosen from virus plants, without regard to the time of inoculation. It was assumed that diseased material was material which was showing symptoms of aucuba mosaic. It soon became evident, however, that the diseased tops fell into two distinct classes as regards their CO_2 output, and it was found that the distinction was associated with the time of inoculation.

This association is not surprising in the light of the results outlined above. It has been shown previously that the amount of symptoms shown by the inoculated plants depends on the amount of growth following inoculation. The more growth the plant has made before inoculation, the greater will be the supply of carbohydrate available to the diseased plant. The stages of development of the inoculated plant must, therefore, be taken into consideration when selecting material for respiration work.

When the data for the CO_2 output of healthy and virus tops are plotted graphically, as has been noted, there are two types of curves. In the first type the CO_2 output of the healthy leaves is higher initially than that of the diseased. In all cases the CO_2 output is expressed in mg. per three-hour period per 10 gm. initial fresh weight. If it be taken on the dry weight (per gm. dry weight) the difference between the virus and healthy tissues is even more pronounced, since the dry-matter content of the healthy plants is much higher than that of the diseased.

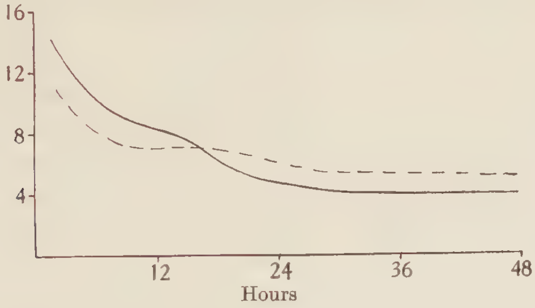
THE RESPIRATION OF PLANTS INOCULATED AS SEEDLINGS.

Initially the CO_2 output of the healthy tops is of the order of 14 mg. of CO_2 per 30 gm. hours, while the CO_2 output of the virus tops is 11 mg. These are representative figures from a large group of experiments. The data are shown graphically in Text-figs. 2 and 3. It may be seen from the figures that the two curves fall steadily at first, though that of the "healthy" CO_2 output falls with a slightly steeper gradient and, consequently, the curves cross at, approximately, the eighteenth hour of the experiment. Thereafter, though both curves continue to fall slowly the "virus" curve remains higher than the healthy. This means that the

CO_2 output of the virus tissue is lower at the early stages of starvation, while at the later stages it is higher than that of the normal tissue.

The advantage of taking three-hourly samples in this experiment and of not running the stream through each tube for twenty-four hours—as has been done in some work on virus respiration—is obvious. Had twenty-four-hour samples been taken the results obtained would have been that the CO_2 output of the healthy was much higher than that of the diseased, despite the fact that at the end of the experimental period the CO_2 output of the virus tissue was actually greater than that of the healthy. It is therefore desirable to take short-period samples continuously for some reasonably long period.

As may be seen from Text-fig. 2, the CO_2 output of both sets of tops tends to fall, that of the healthy at a slightly greater rate than that of the diseased. As a consequence, when the fall in the CO_2 output becomes more gradual, and an "adjusted" state has been reached, the CO_2 output of the diseased tissue is about 6 mg. per 30 gm. hours, while the CO_2 output of the healthy is about 5 mg. per 30 gm. hours. The value of the N.R./D.R. ratio therefore, varies, (N.R. is the CO_2 output of the healthy tissue and D.R. that of the diseased). The value actually was 15/12, later it became 7/7, and latterly 5/6. It is clear, therefore, that some alteration is taking place in the reactions of the two tissues to starvation.



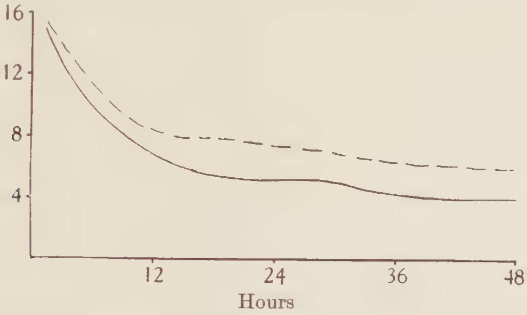
Text-figure 2. CO_2 output in mg. per 10 gm. fresh weight.

An explanation suggests itself if one considers respiration as being controlled by the separate mechanisms. This is the basis of the most recent hypothesis on respiration (cf. Neuberg, Blackman, etc.). The first group of enzymes is responsible for the preparation of the substrate, and the second group of enzymes for the breakdown of the substrate to CO_2, which is measured, and the other products of respiration. It has been shown by Blackman, that the effect of these groups of enzymes can be measured to some extent, and that the two groups are independent, at least, as far as some of their activities are concerned.

If the curves of CO_2 output in this case are examined in the light of the present views on respiration, one or two points emerge. The carbohydrate content of the virus tissue is much lower than that of the healthy. This has been shown by analysis. It is, therefore, quite reasonable to assume that the amount of carbohydrate available immediately to the enzymes of respiration proper, was less in virus than in the healthy tissue. The consequence of this would be that there would be a greater output of CO_2 initially from the healthy tissue. This has been found to be so in the experiments, and the facts are in agreement with the hypothesis. The crossing-over in the curves presents more difficulty, and it is suggested that the greater output of CO_2 from the virus tissue in the later stages of starvation respiration is due to the fact that while the enzymes of respiration proper (*i.e.* those responsible for the breakdown of the substrate with CO_2, etc.) are unaffected by the disease, the enzymes which "activate" the substrate are either rendered more active, or are actually increased in amount in diseased plants. If this were correct, the amount of the less labile carbohydrates of reserve, which were available as a substrate for the respiratory enzymes would be greater in the tissue with the more efficient system—if the amounts in both instances of reserve material were sufficient not to constitute a limiting factor. The hypothesis, nevertheless, fits the facts which are available at present.

THE RESPIRATION OF PLANTS INOCULATED AT LATER STAGES.

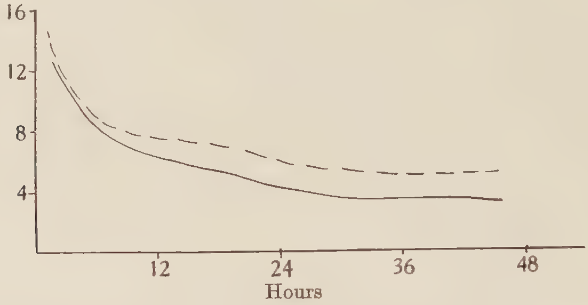
A considerable amount of support is given by the results of another set of experiments on the CO_2 output of healthy and diseased tissues in air. The amount of chlorosis induced by the virus has been shown to depend on the amount of growth subsequent to inoculation. The tops which have just been examined were tops of plants inoculated as seedlings and, therefore, the maximum amount of reduction of chlorophyll had taken place in them. In those tissues, it has been suggested, the reduction in the amount of labile carbohydrates was responsible for the initial low output of CO_2. If, therefore, it were possible to increase this low initial supply of labile carbohydrate, and to have the same stimulation of the enzyme system as appears in diseased tissues, it should be possible to get a larger output of CO_2 from diseased plants initially. The carbohydrate content of diseased tissues, can, as may be gathered from the foregoing, easily be increased by inoculating plants which have developed a few normal leaves. In them the tops will develop symptoms, but a greater supply of carbohydrate will be available for the growing tissues. We have here a tissue which, if our hypothesis is correct, should have an enzyme system of increased efficiency, and a carbohydrate supply almost equal to the healthy plant. It has been shown that the carbohydrate content of the tops of plants inoculated at the fifth or sixth leaf stage, is higher than that of those inoculated as seedlings, and only just lower than that of healthy controls. When such tops are put into the respiration chambers the output of CO_2 is as shown by the curves in Text-fig. 3. The output of the healthy tissue is initially 14 mg., and it falls gradually to 4–5 mg. The output of the diseased tissue is initially 16 mg. and it falls as does the healthy until it is steady at 6–7 mg. The healthy is, therefore, similar to that of the previous experiments, while the output of the virus tissue is consistently higher. The values of the ratio N.R./D.R. at the corresponding times are 14/16, 6/8 and 4·5/6·5. In no case do they reach unity. The value of the N.R./D.R. ratio at the adjusted phase was 5/6 in the first set of experiments and now N.R./D.R. = 5/7, there was, therefore, an excess of output of CO_2 by the tissues when the enzyme efficiency alone was increased by 20 per cent. and by another 20 per cent. when the carbohydrate was in greater amount.



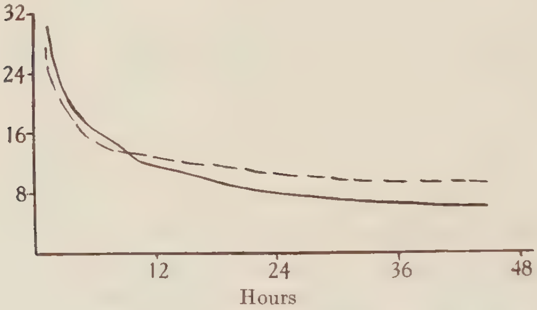
Text-fig. 3. CO_2 output in mg. per 10 gm. fresh weight.

THE OUTPUT OF CO_2 IN NITROGEN.

If the view be justified that the effect of aucuba disease on the tomato plant is to increase the efficiency of that part of the enzyme system which is responsible for "activation" or "glycolysis" or some other part of the reaction which takes place before the breakdown of the substrate to CO_2, etc., then it follows that the breakdown of substrate anaerobically should be increased by the disease. With comparable amounts of carbohydrate, the enzymes of the virus plants should be able to break down the substrate in greater amount than do the enzymes of the healthy controls. This point was examined by estimating the CO_2 output of the tops of healthy plants and of plants which had been inoculated with aucuba at a late stage in their development. The respiring tissues were kept in an atmosphere of nitrogen. As has been seen, the carbohydrate content is but little reduced by late inoculation.



Text-fig. 4. CO_2 output (in nitrogen) in mg. per 10 gm. fresh weight.



Text-fig. 5. CO_2 output (in oxygen) in mg. per 10 gm. fresh weight.

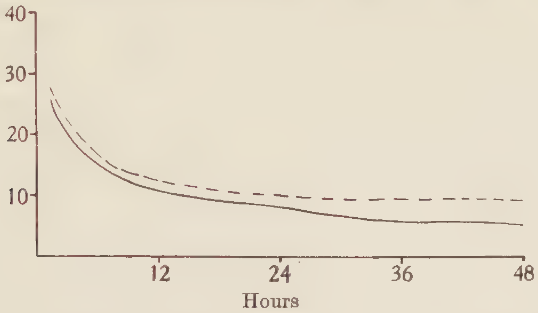
In Text-fig. 4 there are two curves—one representing the CO_2 output of healthy tops kept in the dark in a stream of nitrogen at a constant temperature of 25° C., the other the CO_2 output of virus tissue under similar circumstances. It may be seen that the output in both cases is of the same order initially, but that as soon as the value of the CO_2 output falls off the virus tissues produce more than the healthy. Since the carbohydrate content of both tissues is comparable, and since the absence of oxygen precludes oxidation proper, it would appear that the difference is to be attributed to the more extensive breakdown of the carbohydrate reserves by the more efficient enzyme system. This bears out the hypothesis which was advanced before.

THE OUTPUT OF CO_2 IN OXYGEN.

The output of CO_2 in oxygen is higher from the virus tissue than from the healthy, when tops are taken from healthy plants and from plants inoculated after half-a-dozen leaves had developed. In Text-fig. 5, the curves represent the CO_2 output from the two sets of tissues. Here again the virus tissue has the higher output.

THE OUTPUT OF CO_2 IN TERMS OF THE D.M.C.

It may be argued that the higher CO_2 output from the virus tissues is due, not to the unusual activity of these tissues but to the alteration in the water content or to the alteration in the C/N ratio. In an examination of this point the values of the CO_2 output are recalculated, not in terms of the initial fresh weight but in terms of the dry matter and of the nitrogen content. The expression of the CO_2 output in terms of dry weight and of nitrogen overcomes any objection which may arise by reason of the greater water content of the healthy material, should the conditions of growth of the healthy and of the virus plants be different.

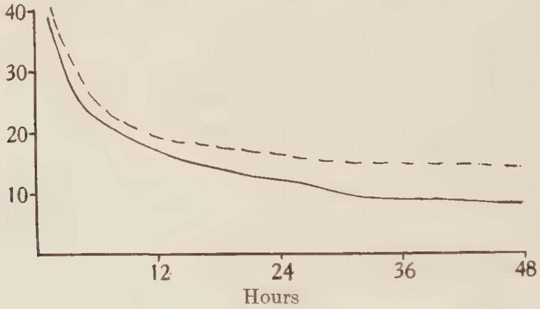


Text-fig. 6. CO_2 output in mg. per gm. dry matter.

In Text-fig. 6 is shown graphically the output of CO_2 from healthy and from virus tissues, expressed in terms of the residual dry matter. This necessitated the drying of the tops after experimentation in an electric oven at 85° C. until two consecutive weights did not vary by more than a milligram. The weight therefore is the dry weight after the respiration measurements are complete—the residual dry matter. The amount of carbohydrate used up in respiration over the period of experiment is slight, however, and no correction has been made for it, since the amounts would be the same, approximately, in both the healthy and the diseased tissue. The graphs of the CO_2 output show that the virus output is again greater than the healthy taken on this basis.

THE OUTPUT OF CO_2 IN TERMS OF THE NITROGEN CONTENT.

The expression of the CO_2 output in terms of residual dry matter obviates any difficulty which might arise from a higher water content of the healthy or virus tissues, but one other consideration may arise. The unusual CO_2 output of the virus tissue might be accounted for by the fact that the protein-carbohydrate content of that tissue had been altered by the disease. It might be argued that the slightly increased nitrogen content of the virus tissues would have the effect of making available a larger amount of protein per unit of carbohydrate and that this made for greater apparent efficiency. Text-fig. 7 illustrates graphically the CO_2 output of the healthy and of the virus tissues when the CO_2 output is expressed in mg. of CO_2 per 100 mg. of nitrogen in the tissue. The nitrogen content was assessed by the Kjeldahl method on the top after the experiment was completed. It will be seen from these curves that the CO_2 output of the virus tissue is higher than that of the healthy per unit of nitrogen, and that the nitrogen content is not the controlling factor.



Text-fig. 7. CO_2 output in mg. per 800 mg. nitrogen.

SUMMARY.

The literature dealing with the effect of virus diseases on the metabolism of the host plants is briefly summarised. Results are presented of work which has been carried out on the aucuba or yellow mosaic in tomato. The effect of time of inoculation has been studied in some detail. It has been found that the plant is generally reduced by the disease, and that the carbohydrate and dry-matter content of the diseased plants is less than that of the controls. The stage of development of the plant is not apparently affected by the disease; the diseased plants, though reduced in size, have the same number of leaves and flower trusses as the controls. The nitrogen content is not materially affected by the disease. The effect of the disease on the respiratory mechanism of the host tissues has been examined, and it has been established that the CO_2 output of these tissues is higher than that of the controls. This is found when the output is expressed in mg. of CO_2 per three-hour period in terms of the initial fresh weight, the residual dry-matter content or of the residual nitrogen content. The higher CO_2 output is also found in respiration in oxygen or in nitrogen. This has been attributed to an increase in the efficiency of the enzyme system of the diseased plants.

This work was carried out under the auspices of the Empire Marketing Board.

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EXPLANATION OF PLATES XI AND XII.

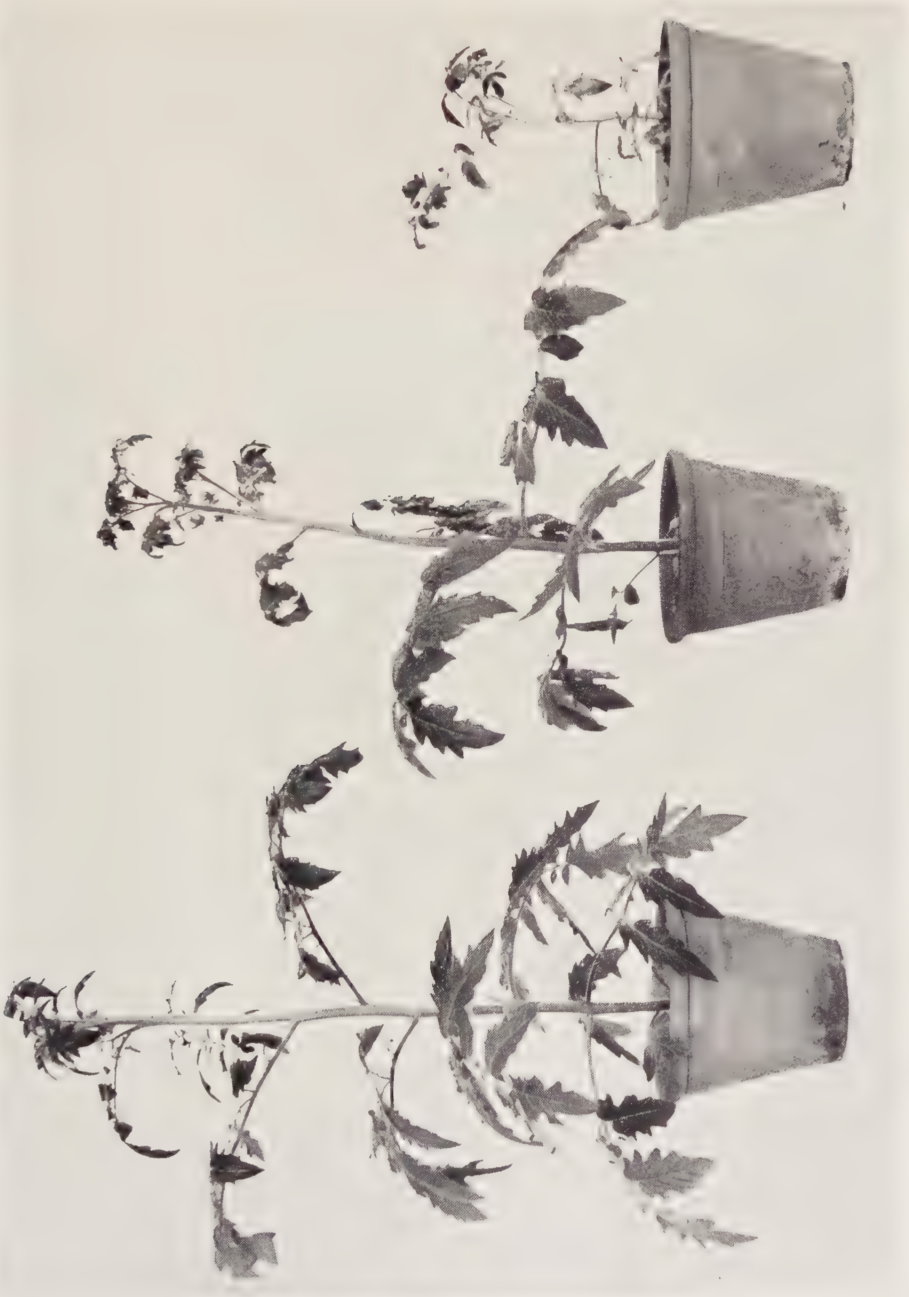
PLATE XI.

The plant on the right was inoculated with Aucuba mosaic as a seedling, the plant on the left is an uninoculated control and the middle plant was inoculated in the 5th leaf stage.

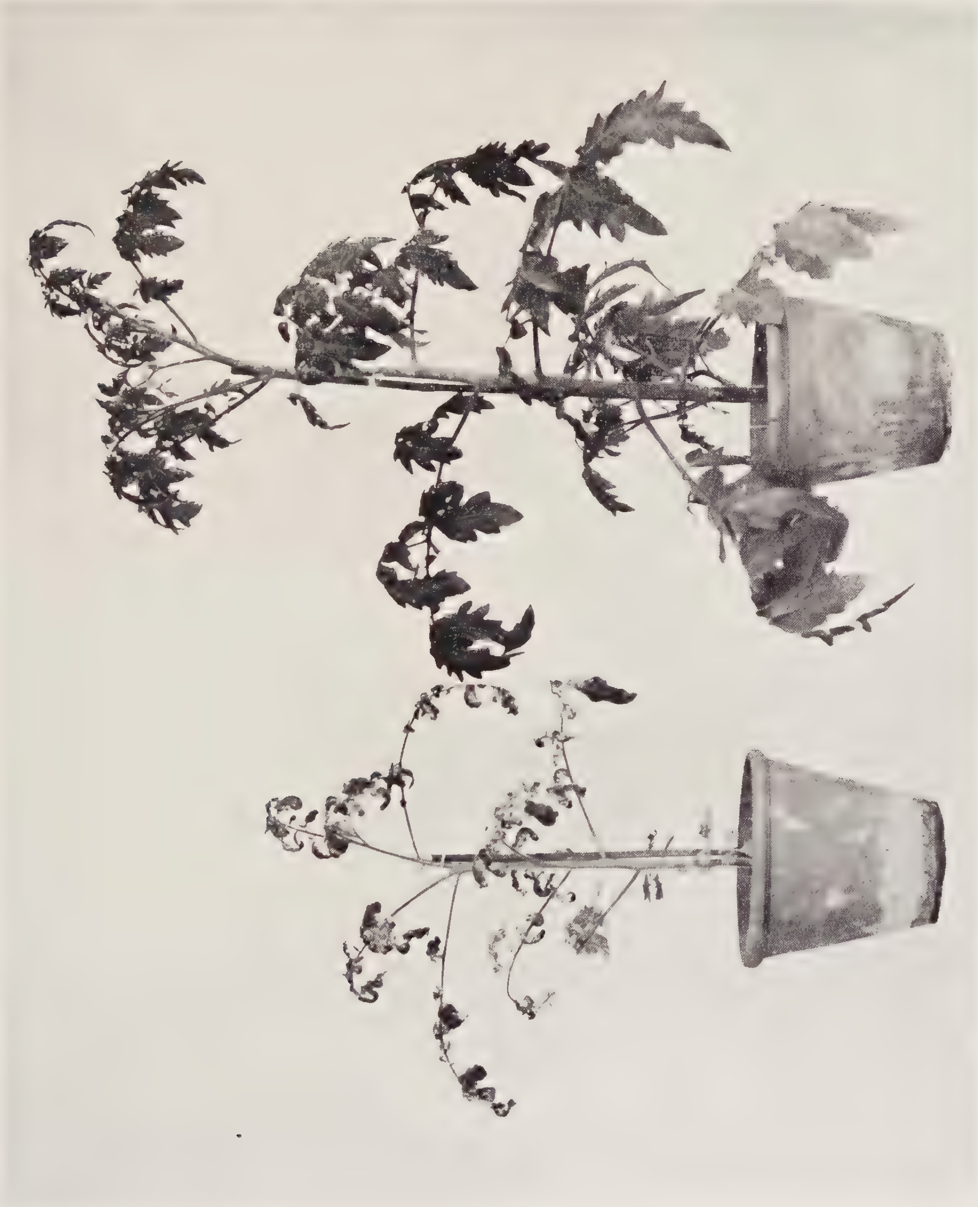
PLATE XII.

The plant on the left was inoculated as a seedling, the plant on the right is a control. The two plants are at the same stage of development.

(*Received October 4th,* 1933.)



CALDWELL.—The Physiology of Virus Diseases in Plants (pp. 206–224).



CALDWELL.—The Physiology of Virus Diseases in Plants (pp. 206–224).

THE EFFECT OF SOAKING IN WATER ON THE "SEEDS" OF SOME GRAMINEAE

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It has been shown in an earlier publication (3) that with cocksfoot (*Dactylis glomerata*) germination was much accelerated if the seeds had previously been soaked in water, and that this action was referable to the effect of such treatment on the pales. Germination was found to be hastened by presoaking in the case of barley and other cereals by Kidd and West (5), and in Germany the soaking of seeds of *Phleum pratense* and *Lolium perenne*, previous to carrying out a germination test, has been officially recommended as being advisable with some samples (6). Tincker (8) found soaked seeds of several species of grasses to germinate better than did untreated samples, and the plants resulting from the former showed a more rapid development. Increases in yield sufficiently large to suggest the economic importance of the treatment were found by Schleh (7) to follow presoaking in oats.

Empirical researches into the way in which acceleration of germination by presoaking is brought about are very infrequent. Such are, however, the work on cocksfoot to which reference has already been made (3) and Harrington's work on freshly harvested seeds of cereals (4). In both these cases acceleration was associated with an increased rate of water absorption.

Although the soaking of grass seeds was formerly carried out frequently by farmers, this procedure is not applied in modern agricultural practice. In consideration of the above results, however, it was thought worth while to determine the effect of presoaking on germination in those species of grass which are commonly employed in agriculture; and also, giving regard to whether the phenomenon shown by cocksfoot, is of general occurrence in the Gramineae.

A method has been described (1) for hastening germination in the field by previous moistening of the seeds, but it differs entirely from the technique considered in this paper.

MATERIAL AND METHODS.

The seeds of cocksfoot and of oats used in the present experiments had been grown in Britain and had been harvested several months; in the case of all the other species, the seeds were commercial samples of the same age.

Unless otherwise stated, in all cases in which treatment was given a routine procedure was followed of soaking the seeds in distilled water, under conditions of good aeration, at a temperature of 22° C. for 17 hours; afterwards drying took place in the laboratory for 24 hours at about 15° C. Subsequent treatment divides the experiments into three groups: (i) the seeds were placed on moist discs of filter paper in covered Petri dishes and kept during germination at a constant temperature of 22° C. in darkness; (ii) with conditions otherwise exactly as in group (i), the temperature of germination fluctuated considerably, about an average of 7° C.; (iii) the seeds were covered to a depth of 6 mm. with fine sterilised soil contained in large covered Petri dishes and having adequate moisture (approximately 30 per cent.).

The seeds exposed on discs of filter paper were regarded as germinated when both root and shoot were visible on each seed, except in the case of *Avena sativa* in which the appearance of the root only was taken as sufficient indication. Germination in the seeds in soil was represented by visible shoots at the surface. All the figures in Tables I–V are averages obtained from three separate lots each of a hundred seeds.

RESULTS.

The germination of soaked and untreated seeds of the several species of grass when set out on discs of filter paper is shown for two widely different temperatures in Table I. A slight acceleration has resulted from presoaking in all species and is more definitely to be observed in the case of *Lolium italicum*, *Festuca pratensis* and *F. rubra*, in which, further, the total germination of the control samples does not quite equal that of the presoaked seeds within the time of duration of the experiment. With *Festuca elatior* and *Alopecurus pratensis*, on the contrary, the relative positions are reversed. A notable feature of the table is, however, the markedly greater acceleration produced by presoaking in seeds germinated at the lower temperature as compared with that obtaining at 22° C. In part, this is what might be expected under any condition which protracted the germination time, since such would increase the absolute value of a difference which remained relatively the

Table I.

Percentage germination of grass seeds on filter paper.

		Temperature variable (average about 7° C.). After				
		7 days	12 days	19 days	26 days	33 days
Lolium perenne	Soaked	—	—	26·0	78·3	90·7
	Control	—	—	20·0	69·7	82·3
L. italicum	Soaked	—	—	58·3	85·0	88·0
	Control	—	—	34·3	75·3	85·7
Phleum pratense	Soaked	42·7	—	62·7	63·0	—
	Control	10·7	—	34·7	35·0	—
Festuca pratensis	Soaked	—	—	7·0	61·0	80·3
	Control	—	—	1·7	43·3	66·7
Cynosurus cristatus	Soaked	—	54·0	88·7	91·0	—
	Control	—	19·7	88·3	94·7	—
Alopecurus pratensis	Soaked	—	—	Nil	11·3	21·7
	Control	—	—	Nil	8·0	22·3
Poa trivialis	Soaked	—	23·3	35·7	45·3	—
	Control	—	4·7	18·3	36·7	—
Festuca rubra	Soaked	—	—	Nil	20·0	33·7
	Control	—	—	Nil	7·3	27·0
Arrhenatherum avenaceum	Soaked	—	—	7·0	73·0	73·3
	Control	—	—	1·0	69·7	73·7
Festuca elatior	Soaked	—	—	Nil	17·0	28·7
	Control	—	—	Nil	16·0	29·7

		Temperature 22° C. After				
		2 days	3 days	4 days	5 days	17 days
Lolium perenne	Soaked	—	33·7	74·7	83·3	85·3
	Control	—	27·7	74·7	84·0	85·7
L. italicum	Soaked	—	30·7	68·3	76·0	89·3
	Control	—	9·0	44·7	61·0	84·0
Phleum pratense	Soaked	43·2	81·5	—	—	96·3
	Control	15·5	90·5	—	—	96·7
Festuca pratensis	Soaked	—	29·0	84·0	94·7	96·7
	Control	—	5·3	64·0	85·3	96·3
Cynosurus cristatus	Soaked	—	—	7·0	15·0	84·7
	Control	—	—	5·0	12·3	78·7
Alopecurus pratensis	Soaked	—	Nil	8·0	16·7	24·7
	Control	—	Nil	2·0	10·3	24·7
Poa trivialis	Soaked	—	—	28·0	35·0	59·0
	Control	—	—	21·0	29·0	68·3
Festuca rubra	Soaked	—	Nil	5·3	22·7	65·3
	Control	—	Nil	Nil	9·3	59·3
Arrhenatherum avenaceum	Soaked	—	3·3	25·3	63·3	79·7
	Control	—	Nil	12·0	54·7	78·0
Festuca elatior	Soaked	—	2·0	36·3	65·7	80·3
	Control	—	Nil	12·0	57·7	86·0

same; but there are very considerable specific variations, this increase in differentiation at the lower temperature not occurring with *Festuca elatior*, *Archenatherum avenaceum* or *Alopecurus pratensis*, being slight with *Lolium italicum*, and remarkably great with *Phleum pratense.* In this last species (and also in the case of *Festuca pratensis* and *Poa trivialis*) germination of normal untreated seeds is very much depressed at low temperatures, as has previously been noted elsewhere (2).

A comparison of the results in Table I, with those obtained from germination tests in soil (cf. Table II), brings out further differences due to altered conditions. When sown in soil containing abundant moisture at a favourably high temperature, the seeds of all the foregoing species derive extremely little benefit from previous soaking in water. This is perhaps to be explained in some measure by the different criterion of germination, seedlings having low vigour of growth being eliminated from the results in the case of soil since they do not appear at the surface. The germination occurring on filter paper is not, however, sufficiently greater than that in soil for this factor to explain the phenomenon entirely.

Table II.

Percentage germination of grass seeds in soil containing 30 *per cent. moisture. Temperature variable* (*average about* 15° C.).

		After					
		5 days	6 days	7 days	9 days	13 days	20 days
Lolium perenne	Soaked	22·3	71·0	81·7	—	—	—
	Control	19·3	73·7	83·7	—	—	—
L. italicum	Soaked	44·0	75·3	83·0	—	—	—
	Control	29·3	70·0	79·0	—	—	—
Phleum pratense	Soaked	—	—	34·7	47·7	56·0	64·0
	Control	—	—	31·0	48·0	58·3	64·0
Festuca pratensis	Soaked	—	43·3	69·0	78·3	—	90·0
	Control	—	23·0	70·0	85·3	—	92·3
Cynosurus cristatus	Soaked	—	—	—	2·7	67·3	87·3
	Control	—	—	—	Nil	67·0	93·7
Alopecurus pratensis	Soaked	—	—	—	21·7	25·7	28·7
	Control	—	—	—	20·3	24·3	27·0
Poa trivialis	Soaked	—	—	—	13·0	28·3	37·0
	Control	—	—	—	5·0	24·0	37·3
Festuca rubra	Soaked	—	—	—	23·0	36·3	54·3
	Control	—	—	—	24·0	39·7	52·7
Arrhenatherum avenaceum	Soaked	—	—	52·3	66·7	68·0	70·0
	Control	—	—	28·7	65·7	68·7	70·3
Festuca elatior	Soaked	—	—	54·3	72·0	77·7	—
	Control	—	—	39·7	67·7	75·3	—
Dactylis glomerata	Soaked	—	—	—	46·0	63·7	73·3
	Control	—	—	—	20·7	38·3	55·3

The relative ineffectiveness of presoaking to alter germination in soil found in these experiments conflicts directly with the results obtained by Tincker in the case of commercial samples of seeds of *Festuca pratensis* and *F. elatior*, and it is not possible to state with certainty the reason for this dissimilarity. In Tincker's experiments soaking took place for the prolonged period of 48 hours; it is not clear at what temperature this occurred or whether the seeds were subsequently dried completely. The most probable explanation of the difference in results is, however, thought to be the higher moisture content and temperature of the soil in the present case. Either of these factors alone is potent to alter greatly the ratio between the respective germinations of soaked and untreated seeds, and the operation of the second of them is discussed elsewhere; the increased differentiation caused by drier soil is explained by the fact that seeds which have been soaked in water retain after air-drying a higher degree of moisture than untreated seeds, and in some cases, also, may absorb water more rapidly than the latter after burial in the soil.

In Table III is shown the germination of *Lolium perenne, Festuca pratensis, F. elatior* and *F. rubra*, in soil of lower moisture content than that already described, but with adequate water for germination. It is apparent that presoaking has considerably accelerated and somewhat increased germination in the first three of these species under such conditions, but there is only slight alteration with *F. rubra* from the behaviour shown in Table II.

Table III.

Percentage germination of grass seeds in soil containing 21 *per cent. moisture. Temperature variable* (*average about* 19° C.).

		After		
		5 days	10 days	26 days
Lolium perenne	Soaked	48·7	80·3	82·3
	Control	29·3	72·0	71·7
Festuca pratensis	Soaked	37·7	95·3	98·0
	Control	7·3	94·0	94·0
F. rubra	Soaked	Nil	20·3	41·3
	Control	Nil	15·3	40·3
F. elatior	Soaked	24·7	75·3	78·0
	Control	7·0	68·0	74·0

Although in many cases an acceleration of germination might result from presoaking, it is clear that the ineffectiveness of such treatment under optimum conditions limits its application in practical agriculture. There is a definite contrast in this respect between the behaviour of the species already described and that of *Dactylis glomerata*, and in demonstration of this, figures for the latter grass (obtained under the same conditions as those for the other species) have been included in Table II.

On account of this pronounced difference in behaviour it would seem to be a legitimate assumption that the mechanisation of acceleration (where such occurs) by presoaking in the case of some or all of the grasses tested in Table I is different from that which has been found in cocksfoot (3). Definite evidence of such a difference has in fact been obtained with *Avena sativa*, the results from which species help somewhat to the understanding of those already described.

Avena sativa resembles most of the foregoing species in showing a greater differentiation between the germination of soaked and untreated seeds at relatively low temperatures. The following experiments have therefore been carried out at temperatures such as occur normally in winter.

In Table IV is shown the effect of presoaking on normal seeds and on caryopses from which the pales have been removed. It is clear that, allowing for an acceleration caused by the latter operation in itself, presoaking has been effective in hastening germination in both cases; its effect, therefore, cannot, as in the case of cocksfoot, operate primarily on the pales. A further difference from the behaviour of *Dactylis glomerata* (and also from that of freshly harvested grains of *Avena sativa*, as described by Harrington (4)), is that the entrance of water into soaked and untreated seeds proceeds at about the same rate in both. Since, also, extreme desiccation after soaking does not remove the beneficial effect of the latter, it is clear that this is independent of the absolute water content of the grains. The acceleration of germination brought about by presoaking does not seem therefore in any way to be associated directly with the water relations of the seed.

Table IV.

Percentage germination of seeds of Avena sativa *on filter paper. Temperature variable* (*average about* 7·5° C.).

		After			
		3 days	4 days	5 days	7 days
Normal seeds	Soaked	5·7	36·7	87·3	99·7
	Control	Nil	Nil	9·0	95·7
Caryopses without pales	Soaked	13·3	64·7	89·7	98·7
	Control	Nil	7·0	86·3	99·3

No evidence has been obtained that gaseous exchange is facilitated in presoaked seeds.

The figures given in Table V show the effect of different periods of soaking (followed in each case by air-drying for 24 hours) at a lower temperature than that of previous experiments. There is a well-marked correlation between the length of time during which soaking has been allowed to occur and the degree of acceleration produced. It would seem therefore that in *Avena sativa* the early metabolic processes of germination are initiated during the period of soaking and that they are merely arrested and not reversed by subsequent drying. So that when conditions suitable to germination again obtain, that process is resumed at about the stage to which it had previously proceeded and the earlier progress represents an advantage, proportional to its length, which the presoaked seeds hold over untreated seeds.

Table V.

Percentage germination of seeds of Avena sativa *on filter paper following different periods of presoaking. Temperature variable* (*average about* 6° C.).

	After			
	6 days	7 days	8 days	9 days
Unsoaked seeds	Nil	1·0	89·0	98·6
Soaked for 6 hours*	Nil	7·3	91·0	97·3
Soaked for 24 hours*	Nil	23·6	89·0	95·7
Soaked for 48 hours†	4·3	62·3	94·7	97·6

* Temperature of soaking about 3° C. † Temperature of soaking about 5° C.

It might be assumed that after a certain prolongation of the soaking period, the subsequent drying of the partially germinated seeds would be detrimental. Such has not, however, been found definitely to take place within any period which did not allow of the visible occurrence of germination[1].

The existence of two such different mechanisms producing the same result in *Avena sativa* and *Dactylis glomerata* confirms the evidence previously obtained that generalisations regarding the effect and operation of soaking cannot be extended over species even closely related—an observation that Kidd and West (5) found to apply also to the effects of presoaking on development subsequent to germination. Assuming, however, the explanation found for *Avena sativa* to apply to some of the other species of Gramineae, the variation which exists between the different grasses as regards the effect of low temperature in increasing the relative advantage of presoaked seeds (cf. Table I) is readily understandable. This advantage would be enhanced particularly in such grasses as at a high temperature pass rapidly through the early processes of germination (*e.g. Phleum pratense*) and also in such (*e.g. Festuca pratensis*) as require a fairly high temperature for them to occur at all.

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[1] In seeds sown in soil a detrimental effect results from soaking for somewhat shorter periods than this.

SUMMARY.

1. The effect of soaking in water on the subsequent germination of several species of Gramineae has been determined under a variety of conditions.

2. With most species an acceleration of germination results from presoaking, but this may be negligibly small under optimum conditions in the soil.

3. Dryness of soil and a relatively low temperature greatly increase the differentiation between the germination of soaked and untreated seeds. A peculiarly extreme case of such an effect by the second of these factors is provided by *Phleum pratense*.

4. It is not possible to generalise regarding the behaviour of different species in relation to soaking in water. Closely related species (*e.g. Lolium italicum* and *L. perenne*, and *Festuca pratensis* and *F. elatior*) show considerable differences of behaviour.

5. In none of the species examined is the acceleration produced by soaking so pronounced as is the case in *Dactylis glomerata*.

6. The acceleration which results from presoaking in *Avena sativa* is brought about by the start given to the early processes of germination.

The author wishes to acknowledge the kindly interest taken in this work by Professor R. G. Stapledon. He is also indebted to Mr R. G. Walker, B.Sc., for carrying out the experiments on *Avena sativa*.

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STUDIES IN BACTERIOSIS

XX. THE SPRAING DISEASE OF POTATO TUBERS

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(With Plates XIII and XIV.)

A consideration of the literature dealing with the different forms of corky lesions occurring in the flesh of potato tubers reveals much confusion. It is only within quite recent times that some order has been brought out of the chaos by the work of Quanjer and his co-workers (16, 17, 18), in Holland, and by Gilbert in America (9). The first-named has distinguished the disease pseudo-netnecrosis, while the latter has further clarified the position by demonstrating clearly the nature and origin of netnecrosis. There remains a complex of tuber flesh necroses which are still in a matter of uncertainty both as to their definition and causes. It is with this group of diseases, comprising those exhibiting symptoms hitherto included under the designation spraing or internal rust spot in England, that the present investigation is concerned. Based on experience with tuber symptoms and on the results of experiments, the conclusion arrived at is, as Horne had already suggested in 1910 (11), that there are two diseases to be distinguished here. It is proposed to use the name spraing[1] for one of them, and the name internal rust spot[2] for the other.

Symptoms of the spraing disease of potato tubers.

Spraing is a disease of the potato tubers alone; there are no manifestations of the disease in the aerial parts of the plant. The disease symptoms are seldom visible on the outside of the tuber, but on cutting affected tubers arc-like lines or rings of suberosed tissue are found which frequently appear to have as their focus or point at which the disease-producing agency entered, either a lenticel or a slight abrasion of the skin of the tuber. The number of such rings is variable, and the focal point is often marked by a browning of the tissue just below a lenticel (Plate XIII, fig. 1). The rings, on occasion, reach almost to the surface of the tuber where, after washing, they become visible to the naked eye (Plate XIII, figs. 3 and 6). Again, short arcs which appear to have no connection with the surface may be found on cutting affected tubers. These arcs form part of a connected system, although the connection is not at first obvious. It seems very probable, as Atanasoff(2) has pointed out, that infection having taken place at a number of points on the tuber surface, interaction has occurred so that the original foci of infection cannot be traced. Sometimes, particularly in certain varieties, larger blotchy areas occur in a tuber, but their relation to the system of arcs can always be traced and they are unlikely to be confused with the haphazard occurrence of the necrotic areas seen in the internal rust spot disease. The photographs in Plate XIII show the disease as it appears in the varieties Kerr's Pink, British Queen and Great Scot. However much it may vary according to the variety it is nevertheless always a typical disease. It corresponds exactly to the Dutch potato disease "Kringerigheid" (Swellengrebel(19)) and the German potato disease "Propfenbildung" (Appel(1)), and was clearly illustrated by Pethybridge(15) in Ireland in 1913.

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[1] The term spraing is a not very commonly used Scottish dialect word and denotes in this connection a form of tuber disease in which misty streak-like marks appear in the flesh. (See Sect. on Spraing, vol. III, *Collected Leaflets on Cultivation and Diseases of Potatoes*, 5th edition, p. 69, Ministry of Agriculture and Fisheries, 1932.)

[2] The term internal rust spot was first used by Paine to denote a condition where the flesh of the potato was spotted with islands of corky tissue of a reddish brown colour. He used the term to include spraing symptoms, but it is now to be used in the sense of the limited definition above.

SYMPTOMS OF THE INTERNAL RUST SPOT DISEASE.

Internal rust spot like spraing is a disease of the tuber alone. The flesh of affected tubers is marked with rusty red to brownish lesions which occur indiscriminately throughout the tissue and appear to form no connected system. The lesions vary in size from mere specks to large irregular blotch-like lesions having a diameter of 1 cm. or more. The number of spots occurring may be very few in slightly affected tubers, but where the disease is strongly developed the rusty coloured areas are numerous and exhibit considerable variation in form and size. The severity of the disease appears to be connected with varietal differences in the tubers. The illustrations given below show the difference between this disease and spraing. That of pseudonetnecrosis, also reproduced here (Plate XIII, fig. 4), shows features which markedly distinguish this disease from both internal rust spot and spraing. Internal rust spot corresponds in some measure to the German disease "Eisenfleckigkeit" as illustrated by Appel (1) in the *Taschenatlas der Kartoffelkrankheiten* and as seen by the present author in Germany.

THEORIES AS TO CAUSE.

When the present investigation was begun three views were current regarding the aetiology of these tuber necroses. Appel (1), and Brandi (3) in Germany, regarded Eisenfleckigkeit as being a non-parasitic disease, and assigned the formation of the internal lesions to bad soil conditions and bad aeration of the growing tubers. Appel stated that in certain soils "cell complexes" in the tuber die owing to the stoppage of the passage of air and the consequent interference with respiration. This explanation however does not seem to be adequate, nor does it fit the facts. More than one worker claims to have shown that there is an infective agency in the soil which causes the formation of necrotic lesions, since tubers grown in sterilised soil do not contract the disease.

Paine (13), working with internal rust spot, as defined earlier in this paper, advanced evidence of the pathogenic nature of an organism named *Bacterium solaniolens*. He was able to produce pronounced local infection at the point of inoculation, and considered it possible that further extension of the disease was only a matter of striking the right conditions. He recorded his failure to isolate *B. solaniolens* from the arc-shaped lesions of spraing as they were at the time described by Horne (11) and Swellengrebel (19). Atanasoff in 1926 (2) repeated these experiments with spraing, but came to the conclusion that the disease appearance produced at the point of inoculation represented simply the usual reaction of the potato tissue, since other bacterial organisms could induce these same conditions, and some even in a more marked way. Atanasoff also showed that spraing (Kringerigheid) could be transmitted by tuber grafting, and adduced other evidence of the infectious nature of this disease. He failed to find any bacterium constantly associated with it, and in his numerous series of attempted isolations of bacteria the tubers proved to be sterile in percentages which ranged from 70 to 90.

Quanjer, in 1926 (16), also stated that he could find no bacterial organism constantly occurring in Kringerigheid lesions. Such organisms as were isolated were found to be non-pathogenic. Burr, in 1928 (4), published an account of his isolation of an organism, named by him *Bacterium rubefaciens*, from material showing "rusty brown lesions in the form of isolated spots, streaks or irregular blotches." Results of infection experiments were given. These experiments were repeated by him in 1931 (6), when he was able to re-isolate the original organism from the experimentally produced lesions. Burr claimed that his organism caused spraing lesions as well as those of the internal rust spot type; but for the following reasons it appears clear that Burr was working mainly with internal rust spot in a rather virulent form:

(1) He states that he found no evidence of the point of infection being a lenticel or abrasion at the surface, and certainly none of his illustrations shows the arcs reaching the surface or the localised point of infection characteristic of spraing.

(2) His inoculation experiments with *B. rubefaciens*, as all his illustrations show, resulted in the formation of internal rust spot lesions occurring indiscriminately in the tissues. There is no indication that the disease produced was spraing. Burr moreover based his conception of "sprain or internal rust spot" disease on the histology of the lesions. Kerling (12) has recently shown that this is of no diagnostic value, since, although the macroscopic appearances are markedly different, no distinguishable microscopic difference can be found between the lesions of spraing and those of pseudonetnecrosis.

(3) Burr's failure to reproduce the disease by tuber grafting seems additional evidence supporting the view that he was not working with spraing to any extent if at all.

(4) The results obtained by Burr indicating that in certain cases transmission of the disease from the seed tuber to the progeny occurs, are not in agreement with those of Frank (7), Swellengrebel (19), Atanasoff (2), Quanjer (16) and Pethybridge (14), (15). Spraing, according to the experience of these workers, is not transmitted to the progeny.

(5) The experience of Burr, that the disease he worked with increased continuously in storage, seems to run counter to that of the above workers, and also to the writer's experience with spraing. Tubers of different varieties typically affected with spraing forwarded from Scotland showed no appreciable increase of the disease after seven months' storage.

From this evidence it may be inferred that Burr was working with internal rust spot, and that his theory of a bacterial cause applies to this disease only, and not to spraing.

Ghirenko (8) in Russia found that internal rust spot (limited according to the writer's conception of the disease, and distinct from spraing) was almost completely absent in soil with a low pH value (4·3–5·8), while it reached its maximum in amount and intensity in soil with a higher pH value (6·0–7·07). He pointed out that the disease seemed to occur at a *p*H value which coincided with the optimum *p*H for growth of bacteria. This inclined him to the view that bacteria may actually be responsible for this disease.

Quanjer, in his 1931 paper (18), definitely considers Kringerigheid (*i.e.* spraing) to be a virus disease, and names it "Concentric Necrosis of the Tuber." The evidence he adduces in support of his view may be summarised as follows: (1) Kringerigheid cannot be ascribed to any cultivable organism. (2) It can be transmitted in its most typical form to healthy tubers by grafting. (3) Infection occurs from the soil, and other viruses are known (*e.g.* mosaic of wheat) in which infection occurs from the soil. (4) There is a strikingly analogous case in the Phony disease of Peach, where the disease is limited to the roots and can be transmitted only by grafting.

When the present investigation began in 1930, it appeared as if the points to be determined were: (1) whether either of the supposedly pathogenic organisms, *B. solaniolens* or *B. rubefaciens*, was constantly associated with the disease; (2) whether either of these organisms could reproduce the disease in all or any of its manifestations; (3) whether graft transmission of the disease, as claimed by Atanasoff, could be confirmed; (4) whether any further evidence as to the infectious nature of the disease could be adduced by attempting to transmit it by inoculation with filtered juice extract of ground up lesions, or by isolating what appeared to be the infectious principle from infected soil. With increasing experience it became evident that, as set out earlier in this paper, there were in effect two distinct diseases to be considered. One of these corresponded exactly with the Dutch potato disease Kringerigheid for which only a virus origin had to be considered. For internal rust spot it remained to be ascertained whether either *B. solaniolens* or *B. rubefaciens* or both were the cause of the disease.

Before passing on to describe the experiments carried out in investigating the four points outlined above and their results, a short account will be given of the theories concerning the mode of infection in both diseases.

THEORIES RELATING TO THE MODE OF INFECTION.

Spraing and internal rust spot both occur on sandy loam types of soil, usually poor in organic matter, potash, and lime, although according to Burr, internal rust spot may occur where the lime content is normal. Infection has been claimed to have been demonstrated by several workers to occur from the soil, and interest has centred on the manner of entry of the parasite. Quanjer (16), Atanasoff (2), and others held that in spraing (Kringerigheid) the causative agent entered at a point or points (usually lenticels or splits in the skin of the tuber) and penetrated for a certain distance without materially altering the tissue. Then, in response to some disturbance or toxic effect, the tissue of the tuber reacted by turning brown. The production of a second or third ring outside the first was explained by supposing that the agent of the disease broke through the brown barriers.

A theory such as this cannot be applied to internal rust spot lesions, and in order to explain their formation Burr (4) postulated the following theory of bacterial invasion: The organism (*B. rubefaciens*) enters through the lenticels of the young tubers or even through the stomata of the stolons before tuberisation, and becomes distributed throughout the watery sap of the intercellular spaces of the tuber. Then, as the tuber matures, the nature of the food supply and the increasing aeration provide conditions for further development of the organism, and disease spots arise indiscriminately in the tissues. At first sight this does not seem possible, but there appears to be no other simple explanation of the presence of bacteria in isolated spots in the tissue of the tuber. A weakness of the theory, as far as the parasitism of the organism *B. rubefaciens* is concerned, is that bacteria other than this species are frequently to be found in small isolated spots in the tuber, and if one does accept Burr's theory one could just as well conclude that any such organism as may be isolated from small internal lesions must have entered through the stomata or lenticels of the young tuber, and therefore has an equal claim with *B. rubefaciens* to be regarded as a causal organism.

Isolation experiments.

The material used in the present investigations was either collected by the writer or was sent to him from different parts of England and Scotland. The varieties worked with were for the most part, Kerr's Pink, British Queen and Great Scot. Both types of disease lesion were used in the following experiments but the results were recorded separately for the "arcs" and "blotches" so that a final analysis of the results of the work was possible for each type.

Technique. The following technique which had been used by Dr Paine in his experiments was found to be the most reliable, comparative tests showing that only a negligible number of contaminations occurred. By means of a sterile knife a shallow cut was made at the surface of a previously externally disinfected tuber, the tuber then being split open by leverage. Necrotic lesions thus exposed were dissected out with small sterile scalpels and the diseased tissue transferred to tubes of prepared media. Check isolations were frequently made in the same way from the interior of healthy tubers. In a number of cases, also, small masses of necrotic tissue were ground up with sterile silver sand in a mortar, a few c.c. of bouillon or soil extract were added and some of the liquid was transferred with a sterile pipette to tubes of media. To duplicate as far as possible the conditions of the experiments of previous workers, use was made of the potato agar and potato-extract media recommended by Paine, as well as of the soil-extract media (using disease-bearing soil from Yorkshire and Surrey) considered necessary by Burr. Generally a duplicate series was made, *i.e.* on splitting open a tuber, half of the lesion was transferred to a tube of potato extract and half (often that part of the necrotic lesion showing on the opposite split surface of the tuber) to a tube of soil extract. Bouillon was also used in a number of isolations.

In these isolation experiments the two types of lesions, "arc" and "blotch," were worked with separately. With internal rust spot the lesions selected for isolation were seldom more than 3 mm. in diameter and frequently less than this. The results of these isolation experiments (totalling upwards of 400) do not in any sense support the idea of bacteria as being the cause of spraing. No organism was found constantly associated with the arc lesions; neither *B. solaniolens* nor *B. rubefaciens* was isolated and, in general, the percentage of organisms isolated was quite low. Isolations from internal rust spot material, on the other hand, showed that it was quite usual for organisms to be present in the lesions. *B. solaniolens*, however, was encountered only once during the whole of these experiments. Organisms closely resembling it but nevertheless differing from it in some essential physiological reactions were found some three or four times. Little success, too, attended the repeated efforts to isolate *B. rubefaciens*. The conditions stated by Burr as to media to be used and the size of the lesions from which isolations were to be made, were complied with. This organism was isolated with certainty only once, but in three other cases organisms resembling *B. rubefaciens* in morphology and in the characteristic motility of that organism, were observed in the culture tubes. Efforts to obtain these organisms in pure culture by plating with gelatine and soil extract agar were, however, unsuccessful. An organism which resembled *B. rubefaciens* morphologically and which also grew very slowly on agar media was obtained in three isolations. Further investigation of this organism, however, showed that it was quite different from *B. rubefaciens.* Its growth on solid media was whitish and it did not liquefy gelatine. *B. rubefaciens* gives a pale yellow growth on agar media and liquefies gelatine fairly rapidly. The isolation of this organism is emphasised, since this occurrence casts some doubt on the statement made above that the organisms which could not be subcultured were possibly *B. rubefaciens.* An analysis of the results failed to show any noticeable selective action of soil extract as against potato extract or bouillon. In the great majority of cases where any organisms at all were found in the culture tubers, as many as two to three different types of bacteria were present. Platings were made from clouded soil-extract tubes on soil-extract agar and gelatine, while from potato extract and bouillon they were made on beef-agar plates. The different colonies appearing on these plates were subcultured; the organisms were obtained in pure culture and their characters noted. Roughly sixty (60) out of the ninety-two (92) organisms isolated during two seasons were different species. Of the remaining thirty-two (32) (which comprised long and short rod types, cocci and cocco-bacilli) three or four occurred some few times, but these appeared to be saprophytic in type and some were spore-formers. No contrast could be drawn between the numbers and types of organisms isolated from lesions in the outer parts of the tuber (cortex) and those isolated from the inner or pith region.

One peculiar fact became evident during the progress of isolation experiments during the 1931–2 season. With increase in age of the diseased tubers kept under dry conditions in the laboratory the number of organisms isolated declined greatly. Taken over a period of one month after digging in August, the average percentage of organisms isolated was as follows: soil extract 40 per cent.; potato extract 50 per cent.; potato agar 40 per cent. Isolations made a month later gave: soil extract 5 per cent.; potato extract nil. One is not inclined to attribute this to any improvement in isolation technique, as from some potatoes low percentages of organisms were obtained in the first week after digging. Thus, in diseased tubers from Haxby (mainly internal rust spot) isolations made two days after the tubers were dug in August gave only 8 per cent. positive isolations. Freshly prepared soil extract (using infected soil from Haxby) was used for these isolations. This early dying out of the organisms in the lesions does not appear to have been experienced by Burr in his work. According to him *B. rubefaciens* can survive in necrotic spots for some months.

ISOLATION EXPERIMENTS USING TUBERS GROWN IN INFECTED SOIL, BUT LIFTED BEFORE ANY DISEASE SYMPTOMS BECAME APPARENT.

The object of these experiments was to test the correctness or otherwise of Burr's hypothesis that *B. rubefaciens* enters the young developing tubers through the lenticels, or even through the stomata of the stolons before tuberisation, and that the organisms become distributed throughout the watery sap of the intercellular spaces of the tuber. If the organism is present in the tubers and merely awaiting more favourable conditions to develop further it is reasonable to suppose that it could be isolated. Accordingly tubers were gathered from plants grown in 12-in. pots containing soil in which internal rust spot occurred under natural conditions. No disease symptoms were to be seen at the time of making the isolations. A number of such isolations failed to reveal the presence of *B. rubefaciens* in the tissues.

CULTURAL CHARACTERS OF *B. SOLANIOLENS* AND *B. RUBEFACIENS*.

While the above-described isolation experiments were in progress, a very detailed study was made of *B. solaniolens* and of *B. rubefaciens* with a view to their exact diagnosis in the event of either proving to be the organism causing internal rust spot. The results of this study are not included here, owing to limitations of space, but they are filed at the Imperial College of Science, London.

INOCULATION EXPERIMENTS, 1931–2.

In these inoculation experiments pure cultures of the two suspect organisms, namely *B. solaniolens*, isolated by the writer from Eclipse tubers into which it had been inoculated by Dr Paine in the summer of 1930, and *B. rubefaciens* obtained in 1930 from the Lister Institute, London, were used. The cultures were made in potato extract, and were added to pots of soil in which potato plants were growing. Following Burr's technique the respective cultures were added to the sterile soil at the time of planting, and the pots were watered at intervals of a week with potato-extract cultures. Both *B. rubefaciens* and *B. solaniolens* grew well in potato extract, and as the cultures were prepared in 50 c.c. batches it is considered that soil impregnation with the organisms was adequate. To make sure that the organisms were present in the vicinity of the developing tubers the following two tests were employed in different experiments: (1) When the potato plant was showing reasonably good development the soil was loosened from around the stolons and young tubers and removed. These were then watered with a suspension of the particular organism, and in some cases small pricks with sterile needles were made in the stolons and young tubers. This method of inoculation was also carried out at later stages of the development of the tubers in order to obviate criticism on the ground that infection may occur only late in tuber development. (2) Tubers were planted in 12-in. pots in which the soil at the time of planting occupied only one-third of the pot. This soil was watered with suspensions of the organisms. Then, as the plants grew, soil was packed around them and at the same time fresh cultures of the organisms were added. This was continued until the pots were three-quarters full. Sufficient time was thus given for thorough impregnation of the soil with the organisms concerned. The soil used in these pot experiments was of the typical light sandy type in which the diseases under consideration usually occur; moreover it was obtained from areas (Haxby in Yorkshire and Wisley in Surrey) where the diseases were present in the current season's crops. The soil was sterilised in the autoclave for five hours at 25 lb. per sq. in. pressure. The tubers used in the 1931 experiments comprised the varieties Kerr's Pink, British Queen and Duke of York. Those used in the 1932 series of experiments were Kerr's Pink and Field Marshal. The tubers used were externally disinfected by steeping for an hour in 1 per cent. mercuric chloride; they were then cut in halves and the cut surfaces allowed to suberize. One set of half-tubers was used in the infection experiments while the other set was used in the control plots. Inoculation experiments in 1931 were divided into two sections: (*a*) those carried out under controlled conditions in pots of sterile soil, (*b*) those carried out as field experiments on sandy soil where potatoes had not previously been grown for at least fourteen years. The experiments under this latter heading were located at the Royal Horticultural Society's Gardens, Wisley, and at the Imperial College Field Biological Station at Slough.

1931 *experiments.*

(1) *Pot experiments at Imperial College.*

The sets were planted in May and the crops were harvested in October and examined in November. This allowed ample time for the disease to develop, if it were present at all, since it was known to be present in crops this same season as early as August. The variety of potato used was Duke of York.

The result was that nothing but healthy tubers were obtained in every case (thirty-three from the six inoculated pots, seventeen from the three controls).

(2) *Field experiments at Slough.*

Potato plants growing on a plot of open ground where the soil was of a sandy to gravelly nature were used in this experiment. The method of inoculation by uncovering the tubers and watering them with heavy suspensions of the particular organisms under investigation and then in some cases pricking the surface of the developing stolons and tubers, was used. Here again no disease at all appeared in any of the tubers (fifty-one from the six inoculated pots, fifty-four from the six controls).

(3) *Field experiments at Wisley.*

Potato plants were grown on sandy soil which had not carried a potato crop for at least fourteen years. The variety used was Kerr's Pink. To prevent any possible complication of results by infection with an aphis transmissible disease such as pseudonetnecrosis, the experimental plants were covered with an aphis-proof structure. Inoculations with *B. solaniolens* and *B. rubefaciens* were carried out as in the preceding experiments. Unfortunately, however, both inoculated and control tubers were found to be badly affected with spraing when the crop was examined in September, so that the experiment was a failure from the point of view of the attempt to demonstrate the pathogenicity of either of the inoculating organisms. The experiment is of some value, however, since it has shown that the infectious agent causing spraing, can remain viable in the soil for a period extending up to fourteen years[1]. As noted earlier, the plot of ground used had not carried a potato crop for at least this length of time. The experiment moreover, gives additional confirmation of the fact that infection occurs from the soil alone, since the plants protected from aphides showed just as high a percentage of diseased tubers as those of an experimental plot in the open.

(4) *Pot experiment. Imperial College,* 1931–2.

This experiment was started in November 1931, and the plants were grown in a glasshouse over the winter. Artificial lighting was used to supplement winter daylight. The tubers before planting were treated with ethylene chlorhydrin to eliminate the dormancy period. The main reason for beginning the experiment at this time was to provide material for inoculation with *B. rubefaciens* and *B. solaniolens*, if these organisms could be freshly isolated from tubers affected with internal rust spot or spraing which were under examination at this time. As indicated under the section dealing with isolations, a fresh culture of *B. rubefaciens* was obtained. No success had attended the efforts to isolate *B. solaniolens* from naturally affected material at the time of starting the experiment, and the organism used was one which was re-isolated from the brown corky tissue that had been produced by core-graft inoculation of a healthy tuber. The organism was still pathogenic, judged by the reaction it produced in the tissue of the tuber into which it had been inoculated by Dr Paine. The variety used was Kerr's Pink. The pots were stored for two months after the plants had died down, before the tubers were harvested. The details of the experiment and the results are summarised in Table I.

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[1] Horne recorded the occurrence of spraing on the sandy type of soil at Wisley in 1914. The author's experiment was carried out in practically the same position as in Horne's experiments, but, since potatoes had not been grown on the piece of land used in the above experiment for at least fourteen years, it was thought safe at the time to carry out the experiment there.

Table I.

Pot No.	Inoculating organism	No. of tubers	Result
1	*B. rubefaciens*	6	One tuber showing necrotic spot in the cortex
2	,,	4	Two tubers with spots of necrotic tissue in the cortex
3	,,	5	Tubers all healthy
4	*B. solaniolens*	5	,, ,,
5	,,	4	,, ,,
6	,,	5	,, ,,
7	Control	5	,, ,,
8	,,	6	,, ,,
9	,,	6	,, ,,

(5) *Pot experiment at the Slough Field Biological Station.*

This experiment was begun a month later than the preceding one and the tubers were harvested in May. Negative results were obtained in all cases (forty-one tubers from the six inoculated pots, sixteen from the three control pots).

Consideration of the results obtained shows that little or no evidence has been obtained to show that either *B. solaniolens* or *B. rubefaciens* is the causative agent of internal rust spot. As will be seen from Table I, infection appears to have been produced by *B. rubefaciens* in three tubers out of fifteen, but this evidence is not considered sufficient to be convincing. Moreover, attempts to re-isolate the organism from the affected tubers failed, so that at present the position as to its pathogenicity is still uncertain. It should be stated that the lesions produced in the three tubers were similar to those figured by Burr in his 1928 publication.

Tuber grafting experiments.

Atanasoff in 1926 (2) announced that he had been able to transmit Kringerigheid (*i.e.* spraing) by grafting pieces of diseased tubers to fresh healthy tubers. He makes the following statement: "...when a piece of a naturally infected tuber is grafted to a newly dug or still fresh and healthy tuber, the disease will develop in the healthy tuber in a most typical form within two or three months, and brown blotches and typical rusty brown arcs and rings will appear far away from the graft in the parenchyma of the healthy tuber." In view of the importance of this result, which if confirmed, would indicate the infectious and perhaps virus nature of spraing, it was deemed most desirable to repeat these experiments. This appeared all the more necessary since Burr in conversation with the author had intimated that he had never succeeded in so transmitting the disease with which he worked. Two methods of grafting were used, and good grafts were obtained after some preliminary failures. In the first method the surfaces of diseased and healthy tubers to be grafted were cut plane, and then the two pieces were pressed firmly together and held thus for some days in a clamp. Healthy and, where possible, freshly dug tubers were used for grafting with diseased tubers.

The second method was the core grafting method of Goss (10). This seemed to offer greater possibilities for success than the first method and it was used most frequently in the later experiments. It was also used for grafting experiments on growing tubers, and will be described in more detail in that section. The grafted tubers were kept under normal conditions for two months, and then cut open and examined.

The results with internal rust spot lesions (blotch type) were, without exception, all negative. Fourteen grafts were made, using Kerr's Pink and Great Scot, both by the plane surface and the core method. With spraing (Kringerigheid) eighteen grafts were made, fourteen plane surface, two core and two slices of diseased tissue inserted between two healthy halves, the varieties being Kerr's Pink, British Queen and Great Scot. All gave negative results except three. One of these was a plane surface graft in British Queen where an arc of necrosed tissue developed in the previously healthy tuber ¼ in. below the graft junction. The remaining two were with Great Scot, where a sliver of diseased tissue was placed between two healthy halves. In both cases there was an elongated necrotic area in the previously healthy tissue, ⅛ in. below the graft.

So far as spraing is concerned, therefore, there would appear to be some foundation for the claim of Atanasoff, but it was not found possible consistently to transmit the disease, or to reproduce it in its really typical form, as he apparently was able to do. Some difficulty was experienced, however, in obtaining a supply of the same varieties of healthy potatoes for use in these experiments as those in which the disease occurred naturally, and the author is inclined to ascribe many of the failures to transmit spraing to this fact. It was noticed, indeed, that in those cases where the same variety of healthy tuber as the infected ones was used, the most convincing cases of transmission occurred. The variety King Edward, which does not often suffer from spraing, had to be used for the majority of the grafts.

The total failure to transmit the "blotch" type of lesion, taken in conjunction with other evidence, has inclined the author finally to the view that spraing (arc lesions) and internal rust spot (blotch lesions) should be regarded as distinct diseases[1]. As indicated earlier, Burr also failed to obtain any evidence for transmission of this type of disease by tuber grafting.

ATTEMPTS TO TRANSMIT THE DISEASE BY USING FILTERED JUICE FROM GROUND-UP SPRAING AND INTERNAL RUST SPOT LESIONS.

Quanjer in 1926 (16) stated that he had tried to adduce evidence of the infectious nature of Kringerigheid by adding finely chopped-up pieces of diseased tubers to soil in which potato plants were growing. Negative results were obtained in all cases, and he concluded that the organism or infectious principle had either died out in the necrotic spots or had lost its virulence. The results of Atanasoff on transmission of the disease by tuber grafting, and the confirmation in some measure of these results in the present investigation, showed that the infectious principle was not dead or avirulent. It seemed therefore desirable to carry further the work of Atanasoff, and see whether the disease could be transmitted by unfiltered and filtered juice from ground-up lesions, using the core grafting method. The following procedure was adopted. Necrotic lesions of both "arc" and "blotch" type were dissected out aseptically from diseased tubers, and ground up with sterile sand in mortars, the two types being kept separate. Potato extract, soil extract, and bouillon were variously used in the making of these suspensions. Cylinders of healthy potato tissue were soaked in this unfiltered suspension, and then forced into smaller bore holes cut in healthy potatoes. Some of the suspension had previously also been allowed to stand in the hollow cylinders which were to receive the cores, one end of each hole being plugged. Some of the juice was filtered through a Seitz filter, and further core-graft inoculations made with this filtered juice. Pots in which potato plants were growing were also watered with the unfiltered suspensions.

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[1] Since the return of the author to Australia he has continued his study of these diseases and has found the blotch type there, but so far has not discovered a single case of spraing. Moreover an examination of the Australian literature has shown that only the blotch type of lesion has been met with by other investigators in that country.

The tubers were examined after two months, and in one case there was definite evidence of transmission of the disease. This occurred in a core-grafted tuber using a suspension of the unfiltered ground-up lesions in bouillon. A necrotic spot area was seen to be extending both into the previously healthy tissue of the core, and outwards for a quarter of an inch into the surrounding tissue. It was limited in extent as seen in longitudinal section, and appeared very like the naturally occurring type of arc lesion. The pale starchless zone around the necrotic area was clearly visible. Other inoculated tubers, where unfiltered and Seitz filtered juice was used, failed to show any reaction whatever. Control core-grafted tubers were uniformly healthy. The same difficulty was experienced here of not being able to get healthy tubers of the same variety as the diseased ones. No evidence of infection was obtained in the pots inoculated with unfiltered suspensions.

ATTEMPTS TO EXTRACT AN INFECTIOUS PRINCIPLE FROM SOILS ON WHICH SPRAING AND INTERNAL RUST SPOT NATURALLY OCCUR.

In both of the diseases under consideration evidence has been produced to indicate that infection occurs from the soil. Since a virus origin has been postulated for spraing (including at that time internal rust spot), it follows if this is the case that there must be some infectious principle in the soil, and the following describes an attempt to extract such a principle from it. The work of Webb (20), in the United States, on the soil transmissible mosaic disease of wheat, indicated that there was little possibility of a virus principle being extracted by ordinary leaching processes. This would no doubt equally be the case with soil containing the hypothetical spraing virus. The virus would in all probability be adsorbed on the surface of the soil particles, and ordinary leaching with water would be of no use. It was decided therefore to try the effect of leaching after the soil had been adjusted to different pH values. It appeared possible that at one point in the pH range, the virus might be freed from the soil particles and thus its extraction in solution would be possible. The following means of buffering the soil to various pH values was adopted. Fifty grams of soil which had given rise to tubers affected with spraing were taken and 50 c.c. of tap water added. After shaking, 100 c.c. of buffered solution pH 4·2 (phthalate and NaOH) were added, and the whole thoroughly shaken for a few minutes. The addition of the 100 c.c. of buffered solution altered the pH value of the soil suspension from 6·9 to 5·2. The suspension was then filtered through filter paper, using a Buchner funnel. Part of this filtrate was used for watering pots containing growing potato plants. The rest was then adjusted to pH 7·6, and filtered through a Seitz filter. The sterile filtrate was then added to other pots in which potato plants were growing. The procedure was repeated as above, using buffer solutions of pH 6·0 and 8·0, whereby the pH values of the soil suspensions were changed to 6·4 and 7·4 respectively; and the soil in which potato plants were growing was likewise watered with both Buchner and Seitz filtrates. The above processes were repeated each week and the filtrates added to the respective pots so that if any infectious principle was being extracted it would be well distributed through the soil surrounding the developing tubers. The sterile soil extract filtrates were likewise used for inoculations of healthy tubers using the core bore method.

The results were entirely negative, not one of the thirty-four tubers produced showing any trace of disease. The results of core-graft inoculations with filtered soil extracts were also negative.

From the negative results obtained it does not appear possible that the adsorption of the infectious principle, if present in the soil, to the soil particles had been overcome.

SUMMARY.

1. The disease symptoms generally grouped in England under the designation "spraing or internal rust spot" have been segregated into two types, namely "arc" lesions constituting spraing disease and "blotch" lesions constituting internal rust spot disease.

2. Isolation experiments have been carried out to see if either of the organisms reputed to be the cause of these tuber necroses (*B. solaniolens* and *B. rubefaciens*) were constantly present in the lesions. The results show that neither organism occurs in spraing, but that there is some

slight evidence that bacteria may be concerned in the production of internal rust spot.

3. Inoculation experiments have been carried out with *B. solaniolens* and *B. rubefaciens*. No infection has been produced by *B. solaniolens*, and though some slight evidence of infection with *B. rubefaciens* has been obtained the results are far from convincing.

4. Spraing has been transmitted by tuber grafting in three cases out of eighteen, so that the results of Atanasoff have to that extent been confirmed. Spraing was also transmitted in one instance by a ground-up suspension of the arc lesions. This evidence, together with the absence of any constantly occurring bacterial organism in the lesions, inclines the writer to support Quanjer's view of the virus origin of spraing. Internal rust spot could not be transmitted by tuber grafting. The fact that organisms are more constantly present in the lesions, that *B. rubefaciens* apparently produced internal rust spot in one experiment, and the failure to transmit this disease by grafting suggest that internal rust spot is a distinct disease from spraing, and may possibly be bacterial in origin.

6. Attempts to isolate a possible infectious principle by leaching from soil after having buffered soil suspensions to different pH values have so far led to no success.

ACKNOWLEDGMENTS.

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Finally, the author wishes to express his gratitude for the help and valuable constructive criticism given so generously throughout by Dr S. G. Paine, under whom this investigation was carried out.

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EXPLANATION OF PLATES XIII AND XIV.

PLATE XIII.

Fig. 1. British Queen tuber cut longitudinally showing spraing lesions. The browning just below the lenticels can be seen here.

Fig. 2. Arcs of necrosed tissue running to the surface, and isolated streaks in Kerr's Pink.

Fig. 3. Ring showing at the surface of a Kerr's Pink tuber.

Fig. 4. Great Scot showing a well-defined arc.

Fig. 5. Badly diseased Kerr's Pink tuber. The condition shown here approximates very closely to that in the German "Pfropfenbildung," where the rings come right to the surface.

Fig. 6. Kerr's Pink, showing necrotic lenticels, and surrounding rings of necrotic tissue

Fig. 7. Spraing arcs in British Queen.

Fig. 8. Arc showing at the surface of a British Queen tuber.

PLATE XIV.

Figs. 1, 2, 3. Internal rust spot lesions in Kerr's Pink.

Fig. 4. Pseudonetnecrosis in Kerr's Pink.

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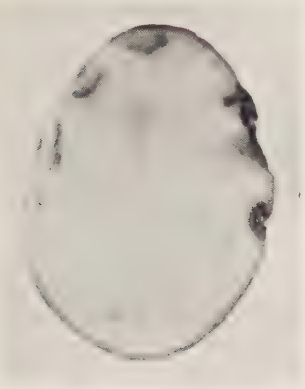

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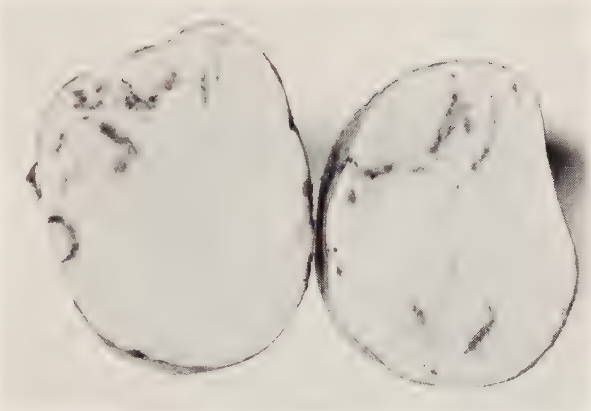

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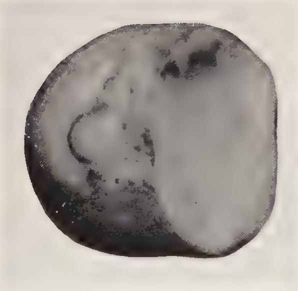

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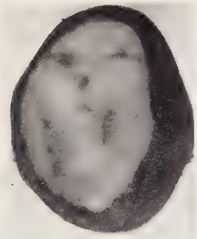

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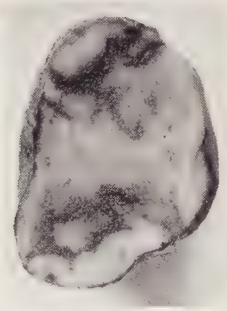

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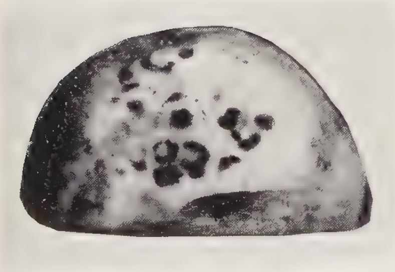

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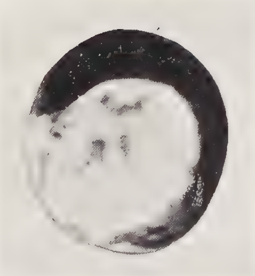
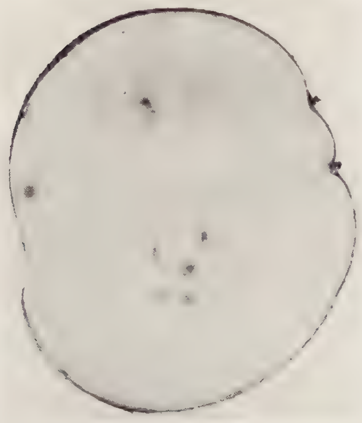


Fig. 7.

Fig. 8.

GRIEVE.—Studies in Bacteriosis (pp. 233–250).



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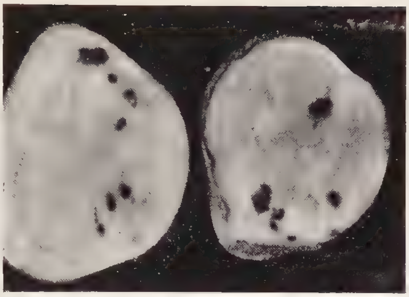

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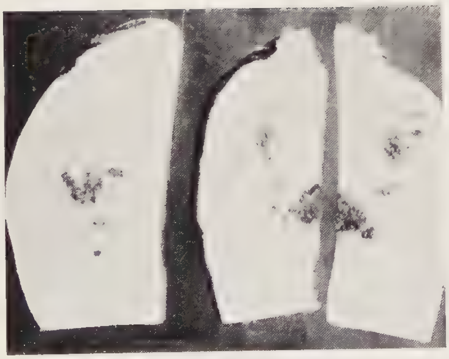

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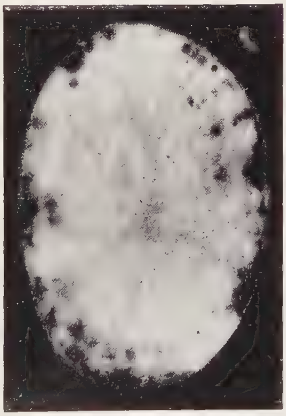

Fig. 4

GRIEVE.—Studies in Bacteriosis (pp. 233–250).

A STUDY OF THE COMPOSITION AND UTILISATION OF ALBERTA PEATS

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NUMEROUS peat or "muskeg" areas are to be found in the wooded soil belt of Alberta, Saskatchewan and Manitoba. The peat areas occur mainly in the northern parts of these provinces. In Manitoba, however, east of the main cultivated wheat area, peat occurs as far south as the United States border; and in Alberta peat areas are found west of the main cultivated area as far south as a point about 200 miles north of the United States boundary. With the information available it is not possible to estimate the extent of the peat areas at all accurately, but it has been estimated that there are 37,000 square miles of peat bogs in Canada and that a large proportion of this land lies in Alberta, Saskatchewan, Manitoba and the adjacent territories (4).

Peat areas consist mainly of organic matter, whereas ordinary soils consist mainly of mineral matter. The peats of Western Canada are usually underlaid by clay or other soil material fine enough to hold the moisture in basins or flats. These basins permitted and encouraged the growth of sphagnum moss and other moisture-loving plants. The accumulation of these plants under water-logged conditions resulted in the formation of peat bogs. The "muskegs" are generally covered by a shrubby growth of Labrador tea (*Ledum groenlandicum*), and frequently there are spruce and tamarack trees growing in the peat areas. Studies of the vegetation of Alberta peat areas have been made by members of the Botany Department of the University of Alberta (7).

Very little experimental work with peats has as yet been done in Western Canada, and we are quite uncertain as to the best methods of reclaiming them or bringing them under cultivation. However, some areas, particularly of shallow peat land, have already been brought under cultivation.

Large areas of peat land have been reclaimed in Denmark, Germany, Sweden and other countries of Europe, and much experimental work has been done at the Bremen peat experimental station, and at other experimental stations in Europe. In England many thousands of acres of "fen" land have been reclaimed. Pioneer work on reclamation of peat land was done in Scotland, and recently experimental work on the reclamation, cultivation and composition of certain Scottish peat lands has been undertaken by the Macaulay Institute for Soil Research (9). Large areas of peat are found in various parts of the United States, and considerable reclamation work has been done in Minnesota and other states. The American Peat Society was formed in 1907.

The Alberta peats studied would be classified as "highmoor" or "moss" peats. Chemical studies of "highmoor" peat profiles of the Eastern United States, including determinations of the organic constituents, have been made by Waksman and Stevens (12).

The following recent work, also, has a bearing upon the experiments reported in the paper. Du Toit and Page (3) have studied the carbon and nitrogen cycles in soil, and the formation of natural humic matter; and Jensen (6) has studied the microbiology and chemistry of farmyard manure decomposition in soil. In both cases some work on decomposition of filter paper cellulose was done. In studies of the biological decomposition of plant materials, Norman (8) found that in general all substances in straw but lignin were attacked by different fungi to a degree relatively proportional to the apparent total loss of organic matter.

The peats of the Canadian prairie provinces generally possess a rather thick surface layer of light-coloured and but slightly decomposed peat. Therefore it will probably require considerable time after drainage to decompose them sufficiently to bring them into a satisfactory condition for the growth of good crops. Application of nitrogen, phosphate and potash fertilisers and farmyard manure will probably prove beneficial. Peats vary in reaction but many of them are very acid, and such peats should respond to treatments with lime, or marl, from local deposits. Their fertiliser requirements may best be determined by analyses, laboratory and greenhouse culture experiments, and field trials.

The underlying layers of peat are darker in colour, decomposed to a greater degree, and probably closer to the productive condition. Therefore, we are mainly concerned with the surface layer which must be decomposed to a considerable degree in order to produce a productive soil.

Preliminary determinations made by the writer at Rothamsted showed that this surface layer was rich in cellulose, and it is believed that this excess of cellulose must be decomposed in order to make the peat productive. While waiting for samples of peat large enough for satisfactory fermentation experiments to come from Canada an experiment on decomposition of relatively pure cellulose was started and carried on. The object was to determine whether the elements commonly applied as fertilisers in field practice (nitrogen, phosphorus and potash) would bring about cellulose decomposition when applied to a comparatively pure form of cellulose. The cellulose used was ground filter paper which contained very little ash (Whatman No. 1—a 24·0 cm. dia. sheet contains 0·0136 gm. ash).

CELLULOSE FERMENTATION EXPERIMENT.

The treatments were as follows: (1) control, moisture only; (2) nitrogen in the form of ammonium carbonate, $(NH_4)_2CO_3.H_2O$, at the rate of 1·0 g. nitrogen per 100 g. water-free cellulose; (3) nitrogen and phosphorus in the forms of ammonium phosphate $(NH_4)_2HPO_4$, and ammonium carbonate, at the rate of 1 gm. nitrogen and 0·4 gm. phosphorus per 100 gm. water-free cellulose; (4) nitrogen, phosphorus and potassium in the forms of ammonium carbonate, and potassium phosphate, K_2HPO_4, at the rate of 1·0 g. nitrogen, 0·4 g. phosphorus, and 1·0 gm. potassium per 100 gm. water-free cellulose; (5) nitrogen, phosphorus, potassium, magnesium, sulphur, calcium, and iron in the forms of ammonium carbonate, potassium phosphate, magnesium sulphate, $MgSO_4.7H_2O$, calcium carbonate, $CaCO_3$, and ferrous sulphate, $FeSO_4$, at the rate of 1·0 gm. nitrogen, 0·4 gm. phosphorus, 1·0 gm. potassium, 0·25 gm. magnesium, 0·25 gm. sulphur, 1·0 gm. calcium, and a trace of iron per 100 gm. water-free cellulose.

Conical flasks of 750 c.c. capacity were used, and there were five treatments in quadruplicate. Fifty grams of water-free cellulose were placed in each, and enough water was added to bring the moisture content up to 80 per cent. of the moisture-holding capacity of the cellulose. No more moisture was added during the incubation period, as the cultures lost moisture very slowly. Aeration was provided by placing cotton-wool in the holes cut through the rubber stoppers. Each culture was then inoculated with a small quantity of water extract from a fertile soil to make sure that sufficient micro-organisms were present. The cultures were incubated in a warm room at a temperature of about 30° C.

At the end of the incubation period the *p*H values of the different cultures were approximately as follows: first *p*H 7·0–7·5; second *p*H 9·5; third *p*H 8·0; fourth *p*H 8·0; fifth *p*H 7·0–7·5. These reactions are satisfactory for microbiological activity.

After 45 days' incubation and again after 107 days' incubation duplicate flasks of each treatment were removed and the residues placed on a Buchner funnel covered with poplin cloth, and washed with hot water. The remaining insoluble residues were dried and weighed.

The experimental results given in Table I show very clearly that under the conditions of this experiment (and probably under any condition) the three elements commonly applied in the form of mineral fertilisers in farm practice will not produce rapid decomposition of cellulose. Even after 107 days these elements had produced very little decomposition, whereas the decomposition was rapid in the cultures to which the other "essential" elements were added.

Table I.

Weights of oven-dried solid residues from 50 *gm. cellulose after fermentation.*

Treatment	After 45 days' incubation gm.	After 107 days' incubation gm.
Control	50·0	49·5
Control	50·4	49·5
N	49·5	49·1
N	49·9	49·5
N + P	49·0	44·0
N + P	48·2	43·9
N + P + K	47·4	46·8
N + P + K	46·7	45·5
N + P + K + Mg + S + Ca + Fe	34·6	19·3
N + P + K + Mg + S + Ca + Fe	33·3	18·5

Repeated attempts were made to determine the numbers of cellulose-decomposing bacteria in these cultures by diluting and plating on cellulose-mineral-agar media, but it was doubtful if the colonies developing on the plates were cellulose decomposers, and satisfactory counts could not be made. It is suggested that the rapid decomposition of cellulose in the cultures to which all "essential" elements were added was brought about mainly by fungi.

At the end of the longer incubation period (after 107 days) the cultures were plated out on a medium which would encourage the growth of a wide variety of ammonifying bacteria. The medium contained 15 gm. agar, 10 gm. peptone, 3 gm. "Lemco" meat extract, and 5 gm. sodium chloride per litre of distilled water. Whether any of the bacteria developing colonies on this medium would be capable of decomposing cellulose is, of course, uncertain. No doubt many of them would be concerned with secondary stages of decomposition.

The relative numbers of bacteria from the different cultures are shown in Table II. Each number was obtained by taking the average of five replicate plates and multiplying by the dilution used. There is no close correlation between the counts shown in Table II and the rates of cellulose decomposition shown in Table I. For example, the addition of nitrogen alone greatly increased the bacterial numbers, but there was no significant destruction or discoloration of the cellulose in this culture. However, it will be observed that the numbers increased with each additional "essential" element, and that the culture to which all "essential" elements were added, and which decomposed rapidly, contained the largest number of bacteria.

Table II.

Plate counts of bacteria in the different cultures at the end of the 107-*day incubation period.*

Culture	No. per gm. of original cellulose
Control	730,000
N	10,160,000
N + P	87,000,000
N + P + K	192,000,000
N + P + K + Mg + S + Ca + Fe	630,000,000

PEAT EXPERIMENTS.

Different horizons or layers of the Alberta peats studied differ greatly in colour or stage of decomposition and in reaction or lime requirement, as is shown below:

Carnwood peat. Surface layer, light in colour, and very acid in reaction (about *p*H 4·0).

Winterburn peat. Surface layer, light in colour and nearly neutral in reaction (*p*H 7·0–7·5). This layer is about 2 ft. thick. Subsurface layer, dark in colour and nearly neutral in reaction (*p*H 7·0–7·5). This layer is about 2 ft. thick. Deep layer, very dark in colour and nearly neutral in reaction (about *p*H 6·5). This layer lies just above the clay underlying this peat area.

Spruce Grove peat. Surface layer, very light in colour and very acid in reaction (about *p*H 4·0). Subsurface layer, light in colour, and nearly neutral in reaction (*p*H 7·0–7·5).

Stonyplain peat. Surface layer, light in colour, and nearly neutral in reaction (*p*H 7·0–7·5). This layer is about 2 ft. thick. Subsurface layer, darker than surface in colour and somewhat acid in reaction (about *p*H 5·5). This sample was taken at a depth of 4 ft.

Ash. Widely different amounts of ash were obtained on ashing these various samples of Alberta peat, as shown in Table III. The surface samples usually contain less ash than the deeper samples because the surface samples are less decomposed. The Stonyplain peat is exceptional in this respect, but this may be due to marl washed over the surface, because it was observed that the surface sample was neutral in reaction and the deep sample somewhat acid. The very high ash content of the deepest sample of Winterburn peat is probably caused by an admixture of underlying clay with the peat.

Table III.

Composition of peat samples (water-free basis).

Peat sample	Total ash %	Cellulose (minus ash) %	Lignin (minus ash) %	Cellulose + lignin + ash %	Ether extract %	Nitrogen N %	Phosphorus P_2O_5 %	Calcium CaO %	Reaction *p*H
Carnwood, 1st depth	4·1	26·0	35·6	65·7	0·7	1·07	0·13	0·90	4·0
Winterburn, 1st depth	7·6	33·5	38·6	79·7	1·1	1·02	0·11	3·43	7·0–7·5
Winterburn, 2nd depth	10·4	5·1	44·1	59·6	0·6	2·54	0·18	3·29	7·0–7·5
Winterburn, 3rd depth	37·5	0·0	42·1	79·6	0·6	1·75	0·27	1·07	6·5
Spruce Grove, 1st depth	14·0	46·9	20·1	81·0	1·1	0·46	0·10	1·19	4·0
Spruce Grove, 2nd depth	20·1	30·2	25·9	76·2	1·0	0·79	0·13	4·61	7·0–7·5
Stonyplain, 1st depth	21·1	21·1	24·6	66·8	0·5	0·89	0·13	4·75	7·0–7·5
Stonyplain, 2nd depth	8·1	11·8	48·9	68·8	0·9	1·74	0·21	3·47	5·5
Vimy, 1st depth	—	54·5*	—	—	—	—	—	—	—
Vimy, 2nd depth	—	11·0*	—	—	—	—	—	—	—

* Including cellulose ash.

Nitrogen. The nitrogen content of these samples of Alberta peat also varies rather widely (Table III). The subsurface layer usually contains about twice as much nitrogen as the surface layer. The explanation for this is that the subsurface layer has been decomposed to a greater degree than the surface layer, and carbon is lost more rapidly than nitrogen on decomposition of raw peat. It is interesting to observe that in ordinary mineral soils the opposite condition holds true: the surface layer nearly always contains more nitrogen than the subsurface because the subsurface contains less organic matter. The nitrogen, calcium and total carbon content of a peat profile obtained near Winterburn, Alberta, was reported by Bedford (2) and in this case, also, the deep sample contained more nitrogen than the surface sample.

It is probable that peats rich in nitrogen will be more quickly reclaimed, or brought into a condition satisfactory for the growth of good crops, than peats poor in nitrogen, other conditions being equal, since a higher nitrogen content usually means a greater degree of decomposition.

Phosphorus. The phosphorus content of the different samples of peat does not vary as much as the nitrogen content (Table III). The subsurface layer in each case contains more phosphorus than the surface, probably because the subsurface is more decomposed.

Peats reclaimed for crop production are frequently deficient in available phosphorus. The availability of the phosphorus in these peat samples has not, as yet, been determined.

Calcium. The calcium content of these samples of Alberta peat varies from less than 1 per cent. to nearly 5 per cent. (Table III).

According to Alway and Nygard (1) peats especially in need of lime narrow down to those with *p*H values of 4·5–3·5, but, according to these investigators, *p*H values do not seem to bring out differences in lime requirements as well as percentages of calcium. Peats which contain 2 per cent. or more of calcium oxide are not likely to require lime for satisfactory crop production.

Judging by the calcium oxide percentages and the *p*H values the Carnwood and Spruce Grove peats require liming, whereas the Winterburn and Stonyplain peats do not require liming for satisfactory crop production.

Organic constituents. Cellulose and lignin form the major part of the organic matter added to soil, and there is usually a good deal more cellulose than lignin in the plant residues added. Cellulose is a hexosan which on hydrolysis yields glucose. Lignin is built up from complex benzene ring groupings, but its chemical structure is not definitely known. Lignin is chemically very resistant, and, by treating organic matter with strong acids other substances are destroyed.

Cellulose was determined by the Jenkins method (5) a convenient modification of the standard one of Cross and Bevan. The Jenkins method, as used for the determination of cellulose in straws, calls for boiling and extracting the straw twice with a solution of approximately 1 per cent. sodium hydroxide, and twice with hydrochloric acid; but it was decided that four successive extractions with alkali and acid were required for the peat samples. The method also calls for chlorinating the straw twice with a definite solution of sodium hypochlorite, each time for a period of 20 min., but it was found necessary to chlorinate the peat samples for several hours instead of 20 min. each time (once overnight as a rule). Possibly fewer extractions with alkali and less chlorination would have been better for certain samples, as some loss of cellulose may have occurred, but it was decided that all samples should be treated alike for purposes of comparison.

Lignin was determined by the method of Ost and Wilkening as, modified by Schwalbe (10). Two grams of the dried material are treated with 60 c.c. of 72 per cent. sulphuric acid and 15 c.c. of 18 per cent. hydrochloric acid and kept in an ice-box for 24 hours. At the end of this period about 500 c.c. distilled water are added, and the whole is boiled for half an hour to complete the hydrolysis. The product is filtered through paper on a gooch crucible, washed, dried, weighed and ashed. The weight of lignin is given by subtracting the weight of ash.

The cellulose content of the peats varies from none to about 47 per cent. of ash-free cellulose (Table III). In the four cases in which comparisons of surface and lower layers of peat can be made, the surface layer contains more cellulose than the lower horizons; and, in the case of the Winterburn peat, in which three depths can be compared, the second depth contains much less than the surface layer, and the dark coloured, deep third layer has lost all of its cellulose. It will be observed that the sample which has the highest percentage of ash-free cellulose has the lowest percentage of lignin. It will also be observed that the three samples which contain more than 40 per cent. of lignin are the three lowest in cellulose. A decrease in cellulose content is therefore usually accompanied by an increase in per cent. lignin. This is to be expected if we assume that when plant residues decompose, the cellulose decomposes more readily than the lignin, the latter being relatively resistant to decomposition.

The cellulose determined might be defined as Cross and Bevan cellulose, since the Jenkins method gives results which are practically identical with those obtained by the Cross and Bevan method. Some hemicellulose may be retained, but, as previously suggested, some loss of cellulose may have occurred also. The percentages of cellulose found in the surface horizons of the Spruce Grove and Vimy samples are very high.

It will be observed that cellulose lignin, and ash together make up about two-thirds or more of the weight of the peat. In the one case in which the fraction is smaller (the Winterburn second depth sample), the percentage of nitrogen or nitrogenous organic matter, is exceptionally high.

The remaining fraction of the peat would be made up of a variety of substances. Nitrogenous organic matter would account for about 3 to 16 per cent. of the total dry weight of the peat, if we assume that the nitrogenous organic matter contains about 16 per cent. of nitrogen. Hemicelluloses were not determined and might account for a considerable percentage of the remaining peat.

The samples were extracted for 5 hours with petroleum ether (boiling-point not higher than 40° C.), in a Soxhlet apparatus, and the extract was evaporated, dried and weighed. However, the material soluble in petroleum ether accounts for only 1 per cent. or less of the total peat (Table III).

EXPERIMENT TO STUDY FERTILISER REQUIREMENTS.

A laboratory peat fermentation experiment was started with the object of studying, in a general way, the fertiliser requirements of a sample of Alberta peat. The surface sample of Carnwood acid peat was used.

Cultures (1) without fertilisers, (2) with nitrogen alone, (3) with phosphorus alone, (4) with nitrogen and phosphorus together, and (5) with nitrogen, phosphorus and potassium together, all with and without lime, were prepared. Each treatment was in duplicate. The fertiliser salts were added in relatively small quantities, comparable to quantities added in field fertiliser practice. Nitrogen was given in the form of ammonium sulphate ($(NH_4)_2SO_4$), phosphorus in the form of calcium acid phosphate ($CaH_4(PO_4)_2—H_2O$), and potassium in the form of potassium sulphate (K_2SO_4), at the rate of 0·1 gm. of each of these three elements per 100 gm. of peat. Lime was added at the rate of 2·0 gm. calcium carbonate per 100 gm. peat.

The peat was ground coarsely and placed in 750 c.c. culture flasks at the rate of 50 gm. water-free peat per flask. The optimum quantity of moisture and the desired salts were added and the cultures were fermented in a warm room at a temperature of approximately 30° C. Aeration was provided by placing cotton-wool in the holes cut through the rubber stoppers, but the cultures were not stirred during the period of incubation.

After incubating the cultures for a period of 94 days, a portion of the peat in each flask was placed in separate pots. The remaining portion of each cultures was left to be plated out for bacterial counts at a later date. Oats were planted in each pot and grown in a greenhouse for a period of 24 days. The young oat plants were then harvested, dried, and weighed. However, the differences in weight were not considered to be significant.

After 110 days' incubation the peat cultures were plated out on a medium which would encourage the growth of ammonifying bacteria. The dilution used was 1 to 100,000, but the numbers of bacteria present proved to be small, averaging less than four colonies per plate. The numbers were too small for satisfactory counts, and they indicate that there was little bacterial activity in the cultures at the end of the incubation period. The number of fungal colonies developing on the plates was greater than the number of bacterial colonies, and it seems probable that fungal activity was greater than bacterial activity.

The growth of the oat seedlings and the bacterial plate counts show that the fertility of this surface peat was not greatly affected by the small additions of fertiliser salts. Possibly a greater effect upon plant growth might have been produced if the seed had been planted at the time of adding the fertiliser salts. This is a question which should be investigated. The experiment indicated that surface peats of this nature with a high percentage of cellulose are not easily decomposed, and suggests that the process of reclamation for satisfactory crop production will require a period of years.

EXPERIMENT TO STUDY THE POSSIBILITY OF BRINGING ABOUT RAPID DECOMPOSITION OF CELLULOSE.

Two laboratory peat fermentation experiments were started with the object of studying the possibility of bringing about rapid decomposition of cellulose in samples of Alberta peat. The acid surface sample of Carnwood peat, and the nearly neutral surface and subsurface samples of Winterburn peat were used.

Cultures (1) without added fertiliser, (2) with nitrogen, phosphorus and potassium, and (3) with nitrogen, phosphorus, potassium and lime, were prepared. Nitrogen and phosphorus were added in the form of ammonium phosphate ($(NH_4)_2HPO_4$). Potassium was added in the form of potassium hydroxide (KOH), in order to decrease the acidity of the acid peat, and lime was added in the form of powdered calcium carbonate ($CaCO_3$). The fertiliser salts were added in relatively large quantities since nitrogen, phosphorus and potassium were each added at the rate of about 2 gm. and calcium carbonate at the rate of 4 gm. per 100 gm. water-free peat.

The peat was coarsely ground and placed in 750 c.c. culture flasks at the rate of 25 gm. water-free peat in each flask. The optimum quantities of moisture and the desired salts were added, and the cultures were fermented at a temperature of approximately 30° C. Aeration was provided by plugging the flasks with cotton-wool, and by stirring the contents with a glass rod every second day. Moisture was added as required to maintain optimum conditions.

After several days' incubation the cultures were tested for hydrogen-ion concentration, and gave the following values: Carnwood surface peat with moisture only, pH 4·0, and with moisture and salts, pH 6·5; Winterburn surface peat with moisture only, pH 6·5, and with added salts pH 8; Winterburn subsurface peat with moisture only pH 6·5, and with added salts, pH 7·5.

At the end of 50 days the percentages of cellulose in the incubated samples were determined (Table IV). In the case of the Carnwood surface peat an appreciable loss of cellulose had occurred in the cultures to which fertiliser salts had been added, and especially in the cultures to which lime had been added. In the cases of the Winterburn surface and subsurface samples the losses, if any, were within the experimental error of the cellulose determinations.

Table IV.

Peat incubated for 50 days with abundance of salt under moist conditions at a temperature of approximately 30° C.

Treatment	Carnwood surface peat cellulose (minus ash) %	Winterburn surface peat cellulose (minus ash) %	Winterburn subsurface peat cellulose (minus ash) %
Original peat, unincubated	26·0	33·5	5·1
Incubated with moisture only	26·5	33·7	5·4
Incubated with moisture + N + P + K	22·0	33·3	5·2
Incubated with moisture + N + P + K + Ca	19·9	—	—

Table V.

Plate counts of the bacteria in the different cultures of Carnwood surface peat at the end of the 50-day incubation period.

Treatment	No. per gm. of original peat
Incubated with moisture only	29,000,000
Incubated with moisture + N + P + K	66,000,000
Incubated with moisture + N + P + K + Ca	70,000,000

At the end of 50 days the Carnwood peat cultures were plated out on Thornton's medium which restricts the spreading of individual colonies and consequent crowding out of other colonies (11). It will be observed (Table V) that the bacterial numbers were more than doubled by the addition of the fertiliser salts, and, with longer periods of incubation it is likely that much greater differences in loss of cellulose could be measured, corresponding more closely with the relative numbers of bacteria.

A second experiment was started with the object of studying the possibility of bringing about rapid decomposition of cellulose in Alberta peat. The acid surface sample of Spruce Grove peat, which contains a high percentage of cellulose, was used.

Cultures (1) without added fertiliser, (2) with nitrogen, phosphorus, potassium, magnesium and sulphur, and (3) with nitrogen, phosphorus, potassium, magnesium, sulphur and lime were prepared. Nitrogen and phosphorus were added in the form of di-ammonium phosphate ($(NH_4)_2HPO_4$). Potassium was added in the form of potassium hydroxide (KOH), in order to decrease the acidity of the acid peat. Magnesium and sulphur were added in the form of magnesium sulphate ($MgSO_4 . 7H_2O$), and lime in the form of powdered calcium carbonate ($CaCO_3$). The fertiliser salts were given in relatively large quantities, the various elements being added at the following rates per 100 gm. water-free peat: nitrogen 2·0 gm., phosphorus 2·2 gm., potassium 2·5 gm., magnesium 0·5 gm., sulphur 0·6 gm. and calcium carbonate 4·0 gm.

The peat was ground coarsely and placed in 750 c.c. flasks at the rate of 25 gm. water-free peat in each flask. The desired salts were added and the peat was saturated with moisture. Some aeration was provided by plugging the flasks with cotton-wool, and by stirring the contents of each flask every second day with a glass rod. Moisture was added as required to maintain a saturated condition. The cultures were incubated at the relatively high temperature of 55° C. for a period of 19 days.

At the end of this period the cultures were analysed for cellulose, corrections being made for losses in weight of peat (or gains due to the addition of salts). The percentages of cellulose in the peat before and after incubation are shown in Table VI. Losses of cellulose occurred in all of the incubated samples. The largest loss, amounting to nearly 30 per cent. of the cellulose originally present, occurred in the culture to which lime was added in addition to the other nutrient salts.

Table VI.

Spruce Grove surface peat incubated for 19 days with abundance of salt under very moist conditions, at a temperature of 55° C.

Treatment	Cellulose (minus ash)
Original peat, unincubated	46·9
Incubated with moisture only	40·7
Incubated with moisture + N + P + K + Mg + S	38·6
Incubated with moisture + N + P + K + Mg + S + Ca	33·1

From these experimental results it is clear that the peat cellulose is not nearly as readily decomposable as the filter paper cellulose used in the experiment discussed in an earlier section of this paper. The resistance of the peat cellulose to decomposition may be due to protection of cellulose by lignin and other substances, and greater resistance to decomposition of peat cellulose as compared to filter paper cellulose.

DISCUSSION.

The cellulose fermentation experiment described in this paper showed very clearly that under the conditions of this experiment (and probably under any conditions) the three elements commonly applied in the form of mineral fertilisers in farm practice will not produce rapid decomposition of filter paper cellulose. Even after 107 days these elements had produced very little decomposition, whereas decomposition was rapid in the cultures to which the other "essential" elements were added. The importance of adding all "essential" elements to the cultures when testing the ability of different micro-organisms to bring about decomposition of purified forms of organic matter, such as filter paper cellulose, or when studying the course of the decomposition of purified forms of organic matter, is therefore apparent.

The numbers of ammonifying bacteria in the cellulose fermentation cultures, determined by the plate count method, increased with each additional "essential" element or group of "essential" elements. It is suggested, however, that the rapid decomposition of cellulose in the cultures to which all "essential" elements were added was brought about mainly by fungi, because repeated attempts to determine the numbers of cellulose decomposing bacteria in these cultures by plate count methods were unsuccessful. The ammonifying bacteria would probably be very largely concerned with the secondary stages of the decomposition.

Different horizons or layers of the Alberta peats studied differ greatly in colour or stage of decomposition, and in reaction of pH value. The surface samples usually contain less ash than the deeper samples because the surface samples are less decomposed. The subsurface layer usually contains about twice as much nitrogen as the surface layer because carbon is lost more rapidly than nitrogen when newly formed peat decomposes. The nitrogen content of the different samples varies more than the phosphorus content. Peats reclaimed for crop production are frequently deficient in available phosphorus, nitrogen and potassium. The calcium oxide percentages and the pH values indicate that the Carnwood and Spruce Grove peats require liming, and that the Winterburn and Stonyplain peats do not require liming, for satisfactory crop production.

A decrease in cellulose content is usually accompanied by an increase in lignin, and this is to be expected if we assume that when plant residues decompose the cellulose decomposes much more readily than the lignin. Cellulose, lignin, and ash together nearly always make up about two-thirds or more of the weight of the peat, and the remaining fraction would be made up of a variety of substances.

Growth of oat seedlings and bacterial plate counts indicated that the fertility of Carnwood surface peat was not greatly increased or affected by the addition (about three to four months earlier) of ordinary application amounts of fertiliser salts. The experiment indicates that surface peats of this nature with high percentages of cellulose are not easily decomposed, and suggests that the process of reclamation for satisfactory crop production will require a period of years. However, if the fertiliser salts had been applied at the time of planting the seeds their effects upon plant growth might have been greater.

The laboratory experiments conducted with the object of studying the possibility of bringing about rapid decomposition of peat cellulose showed that under favourable conditions, including a sufficient supply of nutrient salts, peat cellulose can be decomposed. It is not nearly as readily decomposable as filter paper cellulose, but it is doubtful if the peat will form a satisfactory soil for common crops until the excess of cellulose has been decomposed. It is well known that the addition to ordinary mineral soils of excessive quantities of organic matter rich in cellulose, such as wheat straw, will reduce the fertility of the soil until the excess of cellulose has been decomposed.

It is suggested that the possibility of utilising Canadian peats which contain an exceptionally high percentage of cellulose for commercial purposes such as production of paper, alcohol, acetic acid, etc., should be investigated.

SUMMARY.

1. The three elements commonly applied in the form of mineral fertilisers in farm practice did not produce rapid decomposition of filter paper cellulose in incubated cultures, whereas the addition of all "essential" elements produced rapid decomposition.

2. Fungi appeared to be more important than bacteria in the decomposition of the filter paper cellulose.

3. The numbers of ammonifying bacteria in cellulose fermentation cultures increased with each additional "essential" element or group of "essential" elements.

4. Different horizons or layers of the Alberta peats studied differ greatly in colour or stage of decomposition and in reaction or *p*H value.

5. The surface samples of peat usually contain less ash than the deeper samples.

6. The nitrogen content of the different samples varies rather widely, and the subsurface layer usually contains about twice as much nitrogen as the surface layer.

7. The total phosphorus content of the different samples does not vary as much as the nitrogen content.

8. The calcium oxide percentages and the *p*H values indicate that the Carnwood and Spruce Grove peats require liming and that the Winterburn and Stonyplain peats do not require liming for satisfactory crop production.

9. The cellulose content of the peats varies from none to about 47 per cent. of ash-free cellulose, and the lignin from about 20 to 49 per cent. A decrease in cellulose content is usually accompanied by an increase in lignin.

10. Cellulose, lignin, and ash together nearly always make up about two-thirds or more of the weight of the peat. Nitrogeneous organic matter would account for about 3–16 per cent., and petroleum-ether-soluble material for only 1 per cent. or less of the total peat.

11. Growth of oat seedlings and bacterial plate counts indicated that the fertility of Carnwood surface peat was not greatly increased or affected by the addition (about three to four months earlier) of ordinary applications of fertiliser salts.

12. At the end of an incubation period of 50 days appreciable losses of cellulose had occurred in the Carnwood peat cultures to which an abundant supply of fertiliser salts had been added; and bacterial numbers were increased by the addition of fertiliser salts. In the case of the Winterburn peat the losses of cellulose, if any, were within the experimental error of the determination.

13. After nineteen days' incubation at a relatively high temperature (55° C.) all of the cultures of Spruce Grove peat showed loss of cellulose, the largest loss occurring in the culture to which lime was given in addition to an abundant supply of the other nutrient salts.

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THE SHEEP BLOWFLY PROBLEM IN NORTH WALES[1]

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I. INTRODUCTION.

SHEEP farming was not an outstanding feature of agriculture in North Wales prior to the sixteenth century, and so it is not surprising that references to attacks by sheep maggot fly are absent from early Welsh writings. Sheep were only kept on a few of the larger farms, but there is no reason to believe that these would be less liable to attack by the "fly" than sheep across the border in England. Among the early writings on English agriculture there are a number of references which indicate that, even then, the sheep was no recently acquired host of the sheep maggot fly due to some change in animal husbandry. Fitzherbert (1534) shows clearly that the symptoms of attack, and even the habits of the fly, were well known. His record is worthy of note:

> If a sheep haue mathes, ye shall percyue it by her bytynge, or fyskynge (fidgeting), or shakyng of her tayle, and most commonlye it is moyste and wete: and if it be nyghe unto the tayle, it is ofte tymes greene and fyled (soiled) with his dounge....

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[1] The Zoological Editor for 1933 (J. W. M.) regrets that, owing to an oversight, the publication of this communication has been seriously delayed. The manuscript was received from the Author on February 15th, 1933.

An equally interesting record is found in the works of Thomas Tusser (1557–85) which is as follows:

> Reward not thy sheep, when ye take off his coat,
> With twitches and patches as broad as a groat.
> Let not such ungentleness happen to thine,
> Lest fly and her gentils do make it to pine.
>
> If sheep or if lamb fall a wriggling with tail,
> Go, by and by, search it whiles help may prevail,
> That barberly handled, I dare thee assure,
> Cast dust in his a–s–, thou hast finished thy cure.

At a later date there is evidence from William Youatt (1834) that sheep dogs were specially trained to detect sheep "struck" with fly, so that they could separate such from the flock. There are many records of treatment in these early writings, but these will not be dealt with here. Sheep maggot fly attack, then, was well known in England at the time that sheep farming became an integral part of agriculture in North Wales. At present, sheep farming is the predominant feature, as is indicated from the Agricultural Statistics (1929), the 670,881 acres under crops and grass in Anglesey, Caernarvon, Denbigh and Flint carry 1,004,782 sheep. Sheep maggot fly is an important pest, and, although the actual loss of sheep by death is comparatively small, the loss in time and labour expended in the daily rounding up, shepherding and the examination of flocks is considerable. In an abnormal year, when up to 30–40 per cent. of the sheep may be struck, the actual loss in live weight becomes appreciable.

The present work was commenced as a study of certain fundamental aspects of the problem in the hope that it would point to lines of progress in combating this pest.

II. Survey of sheep maggot flies

(1) *Species involved.*

The preliminary studies included a survey planned to yield data concerning the species involved, general distribution, incidence of parasitism, etc. In view of the recently modified classification of *Lucilia* (Richards, 1926), it was very desirable that the exact species involved should be ascertained. In 1928, therefore, some seventy-two farms were carefully selected as being widely distributed and representing very varied ecological and geographical conditions. Periodically, samples of larvae, from infested *living* sheep, were taken from these farms. Each sample was placed in a separate cage, containing meat and soil, and the cages were deposited in the insectary. When the larvae in each sample had pupated, the pupae were transferred in soil to separate cardboard boxes each with a glass tube projecting from the side. The flies were then observed shortly after they had emerged, and were killed and mounted for identification. The writer is greatly indebted to Mr J. E. Collin for his valuable assistance in confirming the identification of the earlier batches of flies and for his ready help when any doubtful cases arose. Further thanks are due to Mr O. W. Richards who very kindly gave the writer a complete set of the British species of *Lucilia*, a set invaluable in the identification of these flies. One hundred and eighty-two separate batches of larvae were received from the selected farms at different times during 1928, and these yielded 5622 flies. In every case, except two, all the flies bred out were *Lucilia sericata* Mg. In the two exceptions, *Calliphora erythrocephala* Mg. appeared in numbers very small compared with the *Lucilia sericata*. Enquiries into these two cases showed that they were moderately old infestations, which suggests that *Calliphora erythrocephala* was secondary in attack. In no instance was the latter species the only one bred from larvae taken from *living* sheep. Two instances are noted, however, when this species was bred from larvae attacking a *dead* sheep.

In North Wales, therefore, it is apparent that *Lucilia sericata* is *the* sheep maggot fly[1], for, in addition to the survey of 1928, some 200 batches of larvae obtained from live sheep from many other centres have yielded only this species. MacDougall (1909) showed that *Lucilia sericata* was the predominant species involved in Scotland, but he obtained records of sheep attacked by *Calliphora erythrocephala* alone. It was important to find that the studies on sheep maggot flies in North Wales could be confined to *Lucilia sericata*, and it was of added interest to observe that in this country none of the other species of *Lucilia* have, as yet, turned their attention to sheep.

(2) *Incidence of attack.*

The survey also yielded data concerning the incidence of sheep maggot fly attack in the province, during 1928. In addition, certain farmers have kept daily records of the attacks among the flocks during other years. Since it is not practicable to publish the records relating to all farms concerned, the records from five farms, situated in different parts of the province, representing contrasting conditions, have been selected. The farms chosen are as follows:

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[1] While this paper was in the press an important bulletin on "The Sheep Blowfly Problem in Australia" (*Coun. Sci. Ind. Res.* Pamphlet No. 37, January 1933) has appeared. This bulletin, which deals comprehensively with the various aspects of the blowfly problem, states that in Australia *six* species of *primary* flies, *five* species of *secondary* flies and five species of *tertiary* flies are involved. *Lucilia sericata* Mg. ranks second to *Lucilia cuprina* (Wied) in importance as a *primary* fly.

Farm A. Denbighshire, 300 ft. above sea-level, well wooded. *Flock*: 140 Welsh mountain ewes with 150 lambs, 50 shearling sheep.

Farm B. Denbighshire, 600 ft. above sea-level, open. *Flock*: 430 Welsh ewes and lambs, 415 Southdown × Welsh lambs.

Farm C. North Caernarvon, 150 ft. above sea-level, open. *Flock*: 1000 Welsh ewes and lambs, 180 Southdown × Welsh lambs.

Farm D. South Caernarvon, 150 ft. above sea-level, bleak, exposed. *Flock*: 250 Welsh ewes, 200 Southdown × Welsh lambs, 158 Welsh ewes (Rhiw type), 118 Southdown × Rhiw lambs, 62 Wiltshire × Rhiw lambs.

Farm E. Anglesey, 100 ft. above sea-level, very wooded. *Flock*: 120 Kerry Hill ewes, 20 Southdown ewes, 10 Wiltshire ewes, 100 cross-bred lambs.

The period of sheep maggot infestation (Table I) is from May until October inclusive. The earliest record of attack during 1928–32 was May 5th, 1928. It is uncommon, however, to have many attacks before the last week in May, and in no instance was attack recorded on more than three days in that month. At most farms a small number occurred prior to shearing, which is usually completed before July. The month of August generally yields the maximum number of days on which sheep are "struck"; the highest number on any one farm being twenty-five. In this month, also, the highest number of individual sheep were attacked, despite the fact that at least one dipping is usual in this month (the period for double dipping ranges from July 15th to September 15th). Quite commonly, the infestation is well maintained to the latter half of September. Records in October are few, and the shepherds are surprised if the sheep are attacked after the middle of that month. The latest record, during the years under observation, was October 19th, 1932.

The degree of infestation varies with the situation of the farm, as is apparent at the farms included in Table I. Farms A and E are well wooded, and on these farms the *normal* infestation was 28·6, and 20 attacks per 100 sheep respectively. (It should be pointed out that the number of attacks per 100 sheep does not necessarily mean the percentage of sheep attacked, since in some cases the same sheep may have been attacked on more than one occasion. This, however, was seldom common enough to be noted.) On these well-wooded farms, the high infestations—in 1932 in the case of A and in 1931 in the case of E—were 41·1 and 37·3 attacks per 100 sheep respectively. On the farms B, C and D, enquiries show that 20 attacks per 100 sheep would be regarded as *above normal*,

Table I.

Incidence of sheep maggot fly attacks in North Wales.

			Number of days of infestations and sheep attacked														
			May		June		July		August		September		October		Total		
Farm	Year	Infestation	Days	Attacks	Days	Attacks	Days	Attacks	Days	Attacks	Days	Attacks	Days	Attacks	Days	Attacks	% attacks
A	1928	Normal	1	1	3	6	12	35	12	19	15	35	2	2	45	98	28·6
	1932	Abnormal	0	0	9	20	16	26	23	63	15	28	2	3	65	140	41·1
B	1931	Subnormal	0	0	5	9	3	6	9	14	9	19	0	0	26	48	5·6
	1932	Abnormal	0	0	9	46	5	10	14	100	9	30	1	2	38	188	22·1
C	1931	Subnormal	3	6	6	21	8	27	10	20	6	19	1	2	34	95	8·3
	1932	Subnormal	1	1	5	10	6	13	6	9	2	2	0	0	20	35	2·9
D	1928	Subnormal	1	8	7	9	6	9	1	2	0	0	0	0	15	28	3·5
	1932	Subnormal	1	2	5	8	6	9	6	11	0	0	0	0	18	30	3·8
E	1928	Normal	1	1	5	12	8	20	5	9	6	9	4	4	29	55	22·0
	1931	Abnormal	0	0	9	35	10	22	13	27	17	25	3	3	52	112	37·3

whereas the infestations stated by the shepherds to be *below normal* worked out at 5·6 attacks per 100 sheep; 8·3 and 2·9 per 100 sheep; and 7·0 and 7·5 per 100 sheep respectively. During advisory visits in different parts of North Wales opportunity has been taken of ascertaining the attack which shepherds in various districts regarded as severe. It varies as follows: *Upland districts*—10–15 per cent. of the sheep attacked; *Lowland exposed*—20–25 per cent.; *Lowland wooded*—35–40 per cent.

(3) *Non-parasitism of larvae from living sheep.*

A very significant fact arose in connection with the study of parasitism among larvae received in the survey. It must be noted that these larvae had been taken from *living* sheep and were transferred to the cage in the insectary. Each cage was examined daily. No parasites appeared, and all larvae, save the few damaged ones which on examination proved not parasitised, pupated and produced flies. Similarly, no parasites have been bred out from the batches of larvae taken direct from *living* sheep during 1929–32. This absence of parasitism among these larvae of *Lucilia sericata* must not be taken as an indication that its parasites are not common in the province. *Alysia manducator* was collected in quantity around meat-containing cages placed outside the College Buildings at Bangor. It seems, therefore, that under field conditions *Alysia manducator* is not attracted by the larvae of *Lucilia sericata* present on *living* sheep. Observations have confirmed the views expressed by Altson (1920) that this parasite is attracted primarily to decaying animal matter, and it would appear that the infested living sheep affords no attraction. This apparent absence of parasitism among larvae of *Lucilia sericata* on the living host is, doubtless, an important factor which will mitigate success in biological control of blowflies. The infested sheep is a constant and important source of unparasitised larvae.

III. Life history of *Lucilia sericata* Mg. in the Province.

Observations during 1928–32, both in the field and in the laboratory, have clearly shown that the duration of the different stages of the life history varies considerably with fluctuations in temperature, humidity, presence of water, sugar substances and other factors. Preliminary investigations were, therefore, designed to ascertain the *range* of duration of the different stages under conditions in North Wales rather than to study the exact duration of the life history under constant and specific conditions. While this work was in progress, Cousin (1929 *b*) in France published a series of papers on studies on *Lucilia sericata* under controlled laboratory conditions. In the presence of sufficient food, optimum conditions for respiration, ample moisture, and at a constant temperature of 33° C. (91·4° F.) Cousin showed that the life cycle was as follows:

Average time from hatching to the end of the feeding period of the larvae, 3 days; prepupal stage, 3 days; and the pupal stage, 8 days. Oviposition takes place 8 days after emergence, giving the complete life cycle from egg to egg of 22 days. Since, under field conditions in North Wales, the mean temperature rarely attains 90° F., a retardation in the duration of the life history would be expected.

(1) *Incubation period.*

The incubation period of over 100 batches of eggs has been ascertained at different times throughout the seasons. Out-of-doors and on meat, it has varied from 10 hours to 52 hours with an average of 24 hours. While this may be taken as the average incubation period on carrion at atmospheric temperature, it is clear that when eggs are oviposited on the live sheep, and laid near or in contact with the flesh, the temperature will approximate that of the sheep. This temperature, taken at the rectum, is 102° F., and temperatures taken at different positions in the sheep's wool show that there is a variation from that of the atmosphere in the first $\frac{1}{2}$ in. of wool to 86–99° F. in contact with flesh on the back, while under the legs and in folds the temperature was 102° F. From the site of deposited eggs, 87·8° F. (31° C.) was taken to represent the average. At this constant temperature, and with a relative humidity of 100 per cent., 10 batches of eggs hatched at periods ranging from $8\frac{3}{4}$–$10\frac{1}{4}$ hours, with an average of $9\frac{1}{2}$ hours. A similar incubation period at this temperature and humidity was obtained by Wardle (1930). The period of the incubation is, however, small, and the maximum retardation will not influence the duration of the entire life cycle to any marked degree.

(2) *Larval period.*

The larval period is taken as the interval between hatching and the time when the larvae cease feeding, leave the food, and pass down to the soil. Observations on 50 batches of 100 larvae each, feeding on meat in cages at atmospheric conditions, have been recorded at different times of the year. The duration of the larval period has varied from 5 to 11 days, with an average of 6 days. There was no indication of any lengthening of the larval period as the season advanced, as, for instance, in June, 1929, it varied from 6 to 11 days, while in September, 1929, it varied from 5 to 10 days.

Larvae infesting sheep feed at a higher temperature than those developing on meat. The temperature approximates that of blood heat of the host. At a constant temperature of 102° F., and at 100 per cent. relative humidity, the duration of the larval period varied from 48 to 52 hours, with an average of 50 hours. The rapidity with which an attack develops on sheep, examined daily, indicates that a larval period approximating this order is natural in the field.

Table II.

Growth measurements of larvae of Lucilia sericata *at 102° F., and the effect of starvation upon development.*

	Average measurements of 10 larvae				Effect of subsequent starvation of larvae from same batch of eggs	
Age of larvae	Length of larvae mm.	Max. breadth of larvae mm.	Space between 2 and 3 anterior whorls of bristles mm.	Length of mouth-parts† mm.	% Dead	% Produce flies
New hatch	1·65	0·39	0·15	0·276	100	0
5 hours	2·58	0·54	0·2	0·278	100	0
6 ,,	2·98	0·66	0·22	0·284	100	0
10 ,,	3·35	0·80	0·27	0·283	100	0
12 ,,	4·46	0·92	0·36	0·680	100	0
13 ,,	4·82	0·92	0·42	0·712	100	0
17 ,,	5·50	1·16	0·48	0·714	100	0
20 ,,	6·62	1·37	0·55	0·712	100	0
*24 ,, (*a*)	7·3	1·45	0·72	0·714	76	24
*24 ,, (*b*)	8·5	1·50	0·85	1·402		
27 ,,	9·8	1·88	0·86	1·400	30	70
36 ,,	12·0	2·21	1·05	1·461	4	96
43 ,,	14·5	2·70	1·10	1·444	0	100
Prepupa	14·4	2·75	1·05	1·447	—	—

* Larvae at 24 hours were obviously divided into two groups.

† Length of mouth-parts implies length from apical tooth of the cephalo-pharyngeal skeleton to the posterior dorsal process of the pharyngeal sclerite.

When sheep are "struck," the practice adopted by 75 per cent. of the farmers was such that it prompted a special study of the larval period. The majority of shepherds on capturing an infested sheep, cut away the wool in the neighbourhood of the infestation, and rub out the larvae with the hands or shears, all such larvae falling to the ground. It is assumed that these larvae starve and die when removed from their host. When, however, these larvae were collected, it was found that over 90 per cent. produced flies. It should be appreciated, also, that an infestation on sheep consists of a pure culture of *Lucilia sericata* in the complete absence of any competitive secondary flies such as are found in carrion. Moreover, these larvae will produce flies in close proximity to the flock. It is thus apparent that the practice assists rather than controls the pest. It was very desirable, therefore, to ascertain precisely what stage in development the larvae must attain so that, despite subsequent starvation, they can produce flies. With this in view, newly-hatched larvae were placed on meat in a constant temperature chamber at 102° F. and 100 per cent. relative humidity. Batches of 35 of these larvae were removed at definite periods; 10 of each batch were killed by immersion in hot water, and retained for morphological examination, while the remaining 25 larvae were placed in cages, containing only damp soil, under field conditions. The cages were examined periodically, dead larvae removed, and pupae or flies recorded. The results of these experiments are given in Table II.

Larvae feeding at the temperature of the sheep's body (102° F.) for the following periods (Table II): newly-hatched, 5, 6, 10, 12, 13, 17 and 20 hours failed to survive when subsequently isolated and transferred to soil under field conditions. Similarly, 76 per cent. of the larvae which had fed for 24 hours also died, but 24 per cent. successfully pupated and produced imagines. These, of course, were smaller, but proved capable of normal reproduction. 70 per cent. of the larvae which had fed for 27 hours prior to starvation produced flies, while the remaining 30 per cent. were removed dead. Practically all the larvae which had fed for 36 hours produced flies; only 4 per cent. died. Larvae feeding for 43 and 50 hours all produced imagines. A study of the measurements, particularly the mouth-parts, clearly indicated the precise stage of development that must be reached by the larvae in order, on subsequent starvation, to produce imagines. The length of the mouth-parts of the larvae of different ages are given (column 5); the cephalo-pharyngeal skeleton was measured from the apical tooth to the posterior dorsal process of the pharyngeal sclerite. The measurements, obviously, fall into three groups, viz. 28, 71 and 1·44 mm., each representing an instar. The maximum variation in lengths of the mouth-parts among larvae of the same age was 0·03 mm. The first instar terminated about the 12th hour. The second instar ended at the 24th hour, and specimens of larvae in the series 24 (*b*) showed that moulting was in progress at the 24th hour for the spiracles and mouth-parts of the third instar were superimposed upon those of the second instar. Only those batches which included larvae in the third instar yielded flies (column 7). There was no evidence that larvae of the second instar, on subsequent starvation, could undergo ecdysis, develop and produce flies. All larvae of 27 hours were in the third instar, and 70 per cent. produced flies despite subsequent starvation, while, of older larvae, 100 per cent. matured. From a practical viewpoint, the approximate minimum size of larvae which, on starvation, can produce a fly is important. The mature larvae measured 14·5 mm. in length and 2·75 mm. maximum breadth, and in these experiments larvae measuring only 8·5 mm. in length and 1·5 mm. maximum breadth produced flies. In other words, larvae a little over half-size can, on subsequent starvation, produce flies. In practice, a shepherd would not detect an infestation much before the larvae were 24 hours old, and this would explain why over 90 per cent. of the larvae "rubbed out" by the shepherds produced flies. It is, thus, very essential that the practice of "rubbing out" of maggots before treatment should be discouraged, and that farmers, instead, should be advised to apply a larvicide before cutting away the wool and maggots around the area of infestation.

(3) *Prepupal period.*

The prepupal period is the non-feeding stage following the larval period and prior to pupation. It was difficult to ascertain with certainty when this stage commenced, but usually the larvae left the food and passed into the soil. The mean monthly temperature of the soil (4 in.) during May to September inclusive has varied from 51·0 to 68·9° F., and this variation has had a marked effect on the duration of the prepupal period. It is, therefore, advisable to give the range rather than the average duration of the stage. The prepupal period during the summer months has varied from 3 days to 16 days. Under controlled laboratory conditions, Wardle (1930) found that at a similar range of temperatures the duration varied from 3 to 22 days.

Hibernation. During the preliminary work in 1928 (Davies, 1929) it was noted that larvae received from infested sheep after September 8th did not behave like previous larvae and failed to pupate. Instead, the larvae remained in the prepupal state throughout the winter. They were commonly found at depths from 6 to 12 in. They frequently inhabited an earthen chamber formed by caking of the soil caused by pressure during the movements of the larvae. A certain amount of wandering was also noted, particularly when there was any marked change in soil temperature. The maximum depth to which they travel has not been observed, but larvae buried to a depth of 3½ ft. readily returned to the surface. Prepupae have been removed from soil frozen hard by a temperature of 16° F., and have been shown to be viable. Each winter during 1928–32 this mode of hibernation has been repeated. Not a single instance of pupation was observed among the several thousand hibernating prepupae each winter. Apart from the occasional wanderings, no marked activity occurred among the prepupae until about the middle of March, when they were observed moving towards the surface. This movement takes place when the mean daily soil temperature at 8 in. depth approaches 45° F., and when the temperature of the upper layers (4 in.) commences to be consistently above that of the lower level by a few degrees. The dates at which the majority of the prepupae have migrated to the surface under field conditions in North Wales have been as follows:

1928, March 20–26; 1929, March 18–25; 1930, March 25–31;
1931, March 19–28; 1932, March 16–23.

After these dates the majority of prepupae could be found within $\frac{1}{4}$ in. from the surface. It is evident that under North Wales conditions the *normal* mode of hibernation is in the prepupal stage. There are, doubtless, special instances where a knowledge of this can serve in reducing the initial population of flies in the immediate neighbourhood of sheep runs. Since many farmers adopt the practice of gathering the infested sheep into a small field or fold, the presence of the majority of hibernating prepupae at the surface during mid-March to the end of April renders it possible to adopt such cultural methods as will destroy the prepupae.

The mode of hibernation of *Lucilia sericata* in other sheep-rearing countries varies. In South Africa, according to Smit (1929) the fly passes the winter in all stages except the egg stage, and breeds continuously throughout the year. In New South Wales, Gurney and Woodhill (1926) took *Lucilia sericata* in traps at all seasons of the year. At Dellas, Texas, Bishopp (1916) states that "the species normally passes the winter in the larval and pupal stage." In New Zealand, Miller (1921) records that "in some parts, such as Nelson and North Auckland, maggot flies are liable to strike dirty sheep throughout the winter."

(4) *Pupal period.*

Hibernating prepupae have, first, been observed pupating at surface level during the first week in April, but, more commonly, pupation takes place from mid-April to mid-May. The mean temperature (wet) at surface level during this period has varied from 46 to 51° F., and the duration of the pupal stage of this generation has ranged from 28 to 33 days. During July, with mean temperatures from 56 to 60° F., the pupal period has ranged from 15 to 21 days, whereas in September, with mean temperatures from 53 to 55° F., the pupal period has varied from 18 to 24 days. The observations of Holdaway and Evans (1930) in France suggested that pupation was stimulated by parasitism, since the majority of pupae which they found among hibernating prepupae proved to be parasitised. It was further suggested that in the case of the pupae which did not yield parasites, pupation had been stimulated by unsuccessful oviposition of the parasites. In the present observations (Davies, 1930) negative evidence gave support to this view, since, among several thousands of *non-parasitised* larvae obtained from the living sheep, not a single instance of pupation has been observed among the hibernating prepupae.

(5) *Imaginal period.*

The first generation of flies appears during May, though, each year, a few have emerged during the last week in April. There was no significant difference in the sequence of appearance of males and females. The flies were transferred to large muslin cages (3 × 3 × 4 ft.) containing ample supplies of water, sugar and meat, which were placed out of doors. The unsuitable enclosed conditions of the cage markedly prolonged the life history at this stage. Thus, mating was not observed until the 15th day after emergence, and more commonly a longer period elapsed. There was no attempt at mating until the temperature rose above 70° F., and below 65° F. the flies were sluggish. It was evident, also, that light had an important influence on mating. In the case of about 100 flies, both sexes, kept in a room with its limited lighting, and within a muslin cage with ample food, high humidity, and at 80–90° F., no mating took place over a period of 7 weeks. When, however, this cage was carried into a glasshouse at the same temperature, mating took place almost immediately. Even in these glasshouses during the dull days of winter, when favourable temperature, humidity and nutrition have been provided, mating has depended exclusively upon additional light being given by electric light bulbs. At a constant temperature of 91·4° F., Cousin (1929 *a*) observed mating within 5 or 6 days after emergence.

Oviposition occurs within a day or so after copulation. Under caged conditions, oviposition was not observed when the temperature was below 65° F.

The longevity of the flies under caged conditions ranged from 28 to 89 days. Absence of water from the cages was invariably accompanied by rapid death of the flies.

The *range* of the duration of the life history is summed up as follows:

Incubation, 0·36–0·42 days; Larval period, 2–2·3 days; Prepupal period, 3–16 days (during summer); Pupation, 15–33 days; Imagine (to oviposition) 15–28 days; Total 36–80 days.

Number of generations. Under the conditions of these investigations, four generations have been obtained each year, though the last generation of flies has emerged late in September. It is evident that under the open field conditions the life history would be shorter than under caged conditions, thus the last generation of flies would emerge in time to oviposit on sheep or carrion before they succumb to colder conditions. Wardle (1927) from a study of blowflies caught at traps in England, believed that the number of generations of *Lucilia* spp. to be four.

IV. AGE OF SHEEP AS A FACTOR INFLUENCING ATTACK.

During 1932 fifteen farmers, whose farms are scattered throughout the province, kept daily records of the sheep maggot fly infestations among their flocks, and an examination of these, and records kept during previous years, has yielded information regarding the influence of certain factors upon the infestation. The record sheets provided opportunity for dividing the number of attacks on ewes and the number of attacks on lambs. The results are tabulated in Table III. On seven farms, the infestation was negligible, so that the figures for eight farms are given.

Table III.

Sheep maggot fly attack on ewes and on lambs.

Farm	Total ewes	Total attacks on ewes	Attacks per 100 ewes	Max. No.* of lambs	Total attacks on lambs	Attacks per 100 lambs
A	190	7	3·7	225	134	59·5
B	420	15	3·6	400	163	40·7
C	770	7	0·9	620	27	4·3
D	202	7	3·4	240	24	10·0
E	200	7	3·5	225	49	21·7
F	304	10	3·2	300	73	24·3
G	600	9	1·5	500	56	11·2
H	462	10	2·1	457	25	5·4

* The maximum number of lambs during the season is given; in every case there will be an appreciable decline in the numbers of lambs on the farm as the season advances and the fat lambs are sold.

It is evident from Table III that at each farm there was a considerably greater proportion of the lambs than the ewes attacked. The figures are even more striking when it is appreciated that the number of lambs was reduced as the season advanced, hence the figures given for the attacks per 100 lambs are the minimum. In the case of the ewes the numbers were approximately the same throughout the season. The fact that the ewes were shorn and that the conditions rendered by the short wool are not so conducive to attack is one factor which would contribute towards comparative resistance of the ewes. This is not the complete explanation, however, since this difference in the degree of attack was evident during late August and September, when the wool of ewes is almost as long as that on the lambs. There was no evidence among these flocks that "scouring" was more prevalent among the lambs than among the ewes which, of course, would render the subject more liable to attack.

V. BREED OF SHEEP IN RELATION TO ATTACK.

An endeavour has been made to ascertain the degree of infestation among different breeds. Since it proved impossible to have an equal number of sheep of the same breed grazing in the same area throughout the season, observations have been confined to farms where two or more breeds are kept. The principal breeds were: Improved Welsh, Welsh Mountain, Southdown, Wiltshire, Border Leicester, Kerry Hill and black and white Syrian, while the cross-breds include Southdown × Welsh, Wiltshire × Welsh and Southdown × Kerry Hill. It is difficult to present the figures of infestations owing to the fact that throughout the season there was the periodical sale of sheep, particularly of lambs. Only a descriptive account, therefore, can be given. The susceptibility of Southdown sheep to attack by maggots was frequently evident, and, in the Vale of Clwyd districts, where the Southdown breed is more common, farmers invariably commented on this. Sheep of this breed, when attacked, frequently do not show obvious symptoms until the attack is well advanced—in some cases not until the larvae have spread along the back. This susceptibility of the Southdown also appears in the cross-breds, for on farms where a Southdown ram is used on Welsh ewes the lambs are more subject to attack than are the pure-bred Welsh lambs. So significant is the tendency for Southdown to be attacked, that in one district it is the practice to sell off all the Southdown × Welsh lambs before September—the worst month for attacks in this district, and the Wiltshire × Welsh lambs are retained. On farms where both Southdowns and Welsh sheep were kept, the Southdowns were more frequently attacked by *Lucilia sericata*. This does not indicate the presence of any marked resistance to attack by Welsh sheep. In fact, during the observations, the heaviest attack occurred among a flock of pedigree Welsh Mountain sheep kept on a well-wooded farm. The Wiltshire breed were least attacked, and there was no instance of severe infestation among these sheep. The cross Wiltshire × Welsh is much more common in this province than the pure-bred Wiltshire, and here again there was evidence that they were much less subject to attack. When Wiltshire sheep or the Wiltshire crosses are attacked, they soon become particularly restless, and infested sheep are thus easily detected. The Border Leicester breed is found more commonly in the Flintshire district, and here observations were made on two pure-bred flocks. The flocks were in areas where maggot attack is common. Only very few infestations were recorded in these flocks, whereas sheep of mixed crosses on adjacent farms showed a high percentage of attack. Observations were also made on one small flock of 36 Syrian ewes with 41 lambs, and it was observed that this breed was as liable to attack as the Southdown and Welsh sheep in the main flock.

VI. SUMMARY.

1. Early writings indicate that attack by sheep blowflies was common and well known in the early sixteenth century, prior to the establishment of sheep farming in North Wales.

2. A survey of North Wales has shown that *Lucilia sericata* Mg. is *the* sheep blowfly of the province. In two advanced cases only were larvae of *Calliphora erythrocephala* Mg. present, and then only in small numbers.

3. Sheep are attacked from May until October. The degree of attack as indicated in a summary of seasons regarded as severe was as follows: *Upland districts*—10–15 per cent. of sheep attacked; *Lowland districts exposed*—20–25 per cent.; *Lowland wooded*—35–40 per cent.

4. The complete absence of parasitism among larvae taken from *living* sheep was a significant result of the survey. Such a source of unparasitised larvae will mitigate success in the biological control of blowflies.

5. The life history of *Lucilia sericata* under North Wales conditions has been investigated. Four generations are recorded. The normal mode of hibernation is in the prepupal stage, which hibernates in late September or October and returns to the soil surface from mid-March onwards, when the soil temperature at 8 in. approaches 45° F. and when in the upper layers is slightly higher.

6. Shepherds, normally, "rub out" larvae from infested sheep, assuming that starvation and death results in the soil. Over 90 per cent. of such larvae produced imagines, and such infestations are pure cultures of *Lucilia sericata* Mg. with an absence of competitive secondary species. Experiments, combined with a morphological study, showed that at the temperature of the sheep's body, larvae attain the third instar in 24 hours. They are then only half-size, but can successfully produce imagines despite subsequent starvation. The present practice, therefore, should be changed, and a larvicide applied before the larvae are removed from the wool.

7. There is clear evidence of the greater susceptibility of lambs to attack, and the factors involved are being studied.

8. Observations have been made on the relative susceptibility to attack of six different breeds of sheep and their crosses. The degree of attack ranged from the Wiltshire breed, which is only rarely attacked, to the Southdown breed, which is frequently subject to heavy infestations.

The writer wishes to acknowledge the assistance of the several farmers who kept daily records of the infestation among their flocks during the period of these investigations.

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STUDIES ON APHIDES INFESTING THE POTATO CROP

II. APHIS SURVEY: ITS BEARING UPON THE SELECTION OF DISTRICTS FOR SEED POTATO PRODUCTION

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(With Plates XV and XVI and Six Text-figures.)

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I. Introduction.

The part played by aphides in the spread of certain virus diseases is now generally recognised, and the importance of these insects as vectors of the virus diseases of potatoes, particularly leaf-roll, is fully appreciated. There is, however, a surprising lack of information concerning the biology and ecology, under field conditions, of the particular species of aphides concerned. The object of this paper, therefore, is to present the results of a comparative field study of the aphis population at certain *successful* seed potato producing centres, where there has been no increase in virus infection among the stocks, and also at some *unsuccessful* centres, where the increase in virus infection has been rapid.

The writer has been concerned solely with entomological studies and the relation that the work has to the seed potato and virus problems is determined from, and in conjunction with, the published data of Mr J. F. Currie and Dr T. Whitehead, whose valuable assistance the writer wishes gratefully to acknowledge.

II. DESCRIPTION OF OBSERVATION CENTRES.

The aphis survey has been carried out during 1928–33 at five out of fifteen centres in North Wales which were originally selected as prospective seed potato producing centres. Under the conditions of the scheme (1, 2) the farmers undertook to discard all potatoes already on their farms and to grow only the special stocks provided. The seed provided proved to be particularly free from virus diseases, since, according to Currie (2), in no case did the amount of visible virus infection exceed 0·48 per cent. of the plants. The centres have been under the continuous supervision of Mr Currie, who, in addition to guarding against the introduction of infected stocks, rogues out virus-infected plants, if any, during his three inspections per annum. Three of the centres (*D*, *F*, *G*) have produced Certified Seed Potatoes, in which there has been no increase in virus infection since the stocks were introduced. The two other centres (*I*, *J*) were discarded from the scheme in 1930 owing to the introduction and rapid development of virus diseases which took place. Details of the incidence of virus diseases at the different centres are given in Table I, which is reproduced by kind permission of Mr Currie (2).

Table I.

Incidence of virus diseases among potato stocks at selected centres.

Centre	1928 %	1929 %	1930 %	1931 %	1932 %	1933 %
D	0·15	0·42	0·22	0·22	0·13	0·16
F	0·28	0·26	0·15	0·28	0·08	0·31
G	0·40	0·23	0·23	0·22	0·09	0·19
I	0·31	1·63	33·00	Discarded		
J	0·48	0·38	12·98	Discarded		

Centre D. A successful centre—situated on Holy Island, Anglesey, low-lying, altitude 50 ft., on north-west coast exposed to prevailing north-west wind. A significant feature of this centre is that the foliage of the potato crop rapidly matures. This centre is removed from townships and areas where potatoes are common.

Centre F. A successful centre—situated in South Caernarvon, altitude 500 ft., inland, some six miles from the coast, well wooded. No market gardens or large areas of potatoes in close proximity.

Centre G. A successful centre—also in South Caernarvon, at the apex of the Lleyn peninsula, altitude 50 ft. exposed to north-west prevailing wind. Removed from townships or potato areas.

Centre I. Unsuccessful centre—situated in Denbighshire, ½ mile from the sea coast, altitude 13 ft., exposed. In close proximity to Rhyl and adjacent townships with the associated market gardens.

Centre J. Unsuccessful centre—situated in Flintshire, just above the estuary of the river Dee, altitude 400 ft. Well wooded, but not sheltered. Similar to centre F. Not in close proximity to townships.

III. SPECIES OF APHIDES CONCERNED.

There is now general agreement (4, 5, 6) that *Myzus persicae* Sulz. is the species of aphis which is most generally responsible for the dissemination of the leaf-roll disease of potatoes. Smith (5) has shown that one or two individuals only of *Myzus persicae* are sufficient to transmit leaf-roll from an infected to a healthy plant. Confirmation of this has been obtained by the writer, when, in controlled experiments on other aspects of the aphis problem, each of twelve out of thirteen separate individuals of *Myzus persicae* succeeded in conveying leaf-roll to healthy plants grown from half tubers.

With *Macrosiphum gei* Koch, which is frequently the predominant species infesting potatoes in the field, success in transmitting leaf-roll has only on rare occasions (5, 6, 7, 8) been attained. *Myzus pseudosolani* Theo., which was found locally and never in large numbers, is an uncertain transmitter of leaf-roll (7). *Myzus circumflexus* Buckt., a common species under glass, readily transmits this disease (8), but this species is rarely found on potatoes out of doors.

In the present paper, therefore, the main observations reported will be those concerning *Myzus persicae.*

Identification of the different species in the field.

Detailed descriptions of the aphides found on potatoes are given, with figures, by Theobald (9). In field survey work of the kind described, it is necessary that identification should be carried out *in situ* on the plant in the field. Efficiency in this work is largely a matter of experience obtained by confirmation of the identification in the field by subsequent microscopical examination of the individual aphides in the laboratory. It is possible to attain a high degree of accuracy in field identification, and, in order to assist field workers, a comparative description of the aphides, with photographs, is given.

The main task is that of segregating the two predominant species *Myzus persicae* and *Macrosiphum gei* in all stages of development.

Apterae. In the adult stage *Myzus persicae* (Plate XV, fig. 5, 2 mm.) is smaller than *Macrosiphum gei* (Plate XV, fig. 2, 3–4 mm.). The photographs also indicate the difference in general outline. *Myzus persicae,* with its shorter legs and more flattened body, tends to lie flush with the leaf when feeding. It is sluggish when disturbed. *Macrosiphum gei,* with its longer legs, stands more removed from the leaf surface, and, on being disturbed is particularly active. *Colour:* Apterous *Myzus persicae* may be of varying shades of green, usually an olive green, but pink individuals are not uncommon. *Macrosiphum gei* is never pink and is usually a lighter shade of green with a whitish pubescence. There is generally a dark green line down the middle of the abdomen. *Frontal tubercles:* In *Myzus persicae* they are characteristic of the genus, being very pronounced and converging; in *Macrosiphum gei*, they are not so well developed and are divergent. *Antennae:* Slightly shorter than the body in *Myzus persicae*, longer than the body in *Macrosiphum gei*. *Cornicles:* Notably converging in *Myzus persicae*, and held in the same plane as the body; in *Macrosiphum gei* they are usually parallel or diverging, not swollen, and often carried at right angles to the body. *Cauda:* Bluntly triangular in *Myzus persicae*, not very long; in *Macrosiphum gei*, long, ensiform, protruding upwards at tip.

It is often necessary to identify nymphs and these one day old, are illustrated in Plate XV, fig. 6 (*Myzus persicae*) and fig. 3 (*Macrosiphum gei*). *Myzus persicae* is more pear-shaped in outline, and the converging cornicles are marked. In young nymphs of *Macrosiphum gei* the outline is more rectangular, and the cornicles divergent or parallel. They often protrude at right angles to the body. When disturbed, nymphs of *Macrosiphum gei* are very active. Other comparative characters are similar to those in apterous adults.

Alatae. It is not difficult to distinguish between the alatae of these two species. *Myzus persicae*, Plate XV, fig. 4, has the characteristic converging tubercles; antennae not longer than the body; cornicles converging and slightly swollen. Head, pronotal band and thorax are black, and there is a characteristic dorsal black patch in front of and between the cornicles. The frontal tubercles of *Macrosiphum gei*, Plate XV, fig. 1, on the other hand, are not outstanding; they diverge; antennae are longer than the body; cornicles diverging and cylindrical. Head and thorax are yellowish green; lobes brownish. The abdomen is uniformly light green.

Myzus pseudosolani Theo., which has not been recorded in significant numbers, is not likely to be confused with *Macrosiphum gei* at any stage. *Alatae.* It can readily be distinguished from *Myzus persicae* by its body marks. Its general body colour is green, and the head and thorax are darkened but not black. The black markings of the abdomen are arranged in definite transverse bands, one on each abdominal segment, not in a black patch as in *Myzus persicae*. The cornicles are much paler than in *Myzus persicae*. *Apterae.* The body of *Myzus pseudosolani* is distinctly shiny and appears more spherical; the cornicles are paler.

Mr F. Laing informs me that he considers this species to be identical with that described as *Myzus convolvuli* Kalt.

Another species of aphis that is occasionally found on the leaves of potatoes is *Aphis solanina* Pass. It is readily distinguished from the other species by its deep apple green colour, its smaller size and its tendency to remain in colonies. Normally, it is only rarely found, but in 1933 it was more common.

IV. Infestation of aphides at the different centres.

(1) *Technique.*

It proved necessary to develop a technique for the study of the comparative intensity of the aphis population which was readily applicable under field conditions, and which would give data statistically comparable. It was decided, ultimately, to take the compound potato leaf, with the several leaflets, as the unit for examination. The number of aphides per 100 leaves taken at random as the crop was traversed was regarded as an index of the aphis population at a given centre. The potato crop at the College Farm was used in order to ascertain the accuracy of this method, counts being taken in eight replications of 100 leaves each, the same area being traversed on eight successive occasions. A note was made of the aphis population of each leaf, the species of each individual being recorded. The following data concern *Myzus persicae* only, though the complete records include other species. The number of *M. persicae* per 100 leaves in each of the eight replications was as follows: 26, 38, 36, 34, 33, 46, 42, 44. The probable error of the mean is 1·34. The nature of this distribution, however, can best be obtained by giving the exact record of 100 leaves in the order in which they were obtained. A representative count is selected, and the data given in Text-fig. 1; only *M. persicae* is recorded.

1	1	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0
1	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	1	0	0
1	0	0	0	2	0	0	1	0	0	1	0	0	2	0	1	1	1	0	0
0	0	0	0	1	3	2	0	0	1	0	0	0	0	0	0	1	0	0	0
0	0	1	0	0	0	1	0	0	0	3	0	0	1	0	0	0	0	0	0

Total 37

Text-fig. 1. Sample of infestation of *Myzus persicae* on 100 leaves.

1	12	6	1	3	9	18	20	4	2
10	7	5	5	3	9	42	15	3	23
17	5	4	1	5	18	18	4	13	1
11	6	9	4	33	15	35	10	24	5
6	19	8	11	38	5	8	1	14	11

Total 557

5	5	12	4	12	9	28	3	36	15
8	12	10	7	12	35	5	4	18	5
7	10	21	8	7	5	20	11	18	4
8	6	7	25	12	6	2	25	8	11
13	10	7	5	8	11	2	5	10	3

Total 540

Text-fig. 2. Sample of heavy infestation of *Myzus persicae* on two lots of 50 leaves each taken from same plot.

This (Text-fig. 1) is a sample from a moderate infestation. Unfortunately, it was not possible to make eight replications in a heavy infestation, but the nature of a heavy infestation can be shown from two representative lots of 50 leaves each taken over the same area. The number of *Myzus persicae* found on each leaf is recorded in sequence on Text-fig. 2.

It will be observed that in a moderate infestation (Text-fig. 1), and particularly in a slight infestation, the record of 100 leaves is a reliable approximation to the actual degree of infestation, for in these instances the range of the infestation per leaf is only from one to three individuals, and the frequency with which the aphides occur indicates that the sample is a representative one. With slight infestations, when a rare instance of a large colony of *Myzus persicae* has been found in such environment as a curled leaf, or when reason for non-dispersal of the aphides was apparent, the exceptional figure was not included in the averages for the centre, since it would clearly give misleading results.

In heavy infestations, while it has not been possible to give figures for the probable error of the mean of a number of counts, it is evident from the nature of the figures presented that, for comparative purposes, the single count of 100 leaves, taken at random, is sufficiently reliable as a sample. The fact that the aphides are well distributed over the crop as a whole can be explained by the surprisingly frequent movement of the individual aphides (3). Further, so far as *M. persicae* is concerned, the habit of the alatae of depositing a single nymph and then taking to wing to repeat the process on other potato leaves does account for a general distribution (3).

(2) *The aphis population.*

The observations on the aphis population of the potato crop during 1928 and 1929 were of a preliminary character, and the results have been published (1). It was found that all centres, though chosen for their bleak and exposed conditions, were liable to heavy infestation with aphides. The nature of the infestation differed. In the eastern part of North Wales (I and J) the maximum infestation was reached during mid-July, whereas at the western centres, particularly F and G, it was delayed until about mid-August. Both species—*Myzus persicae* and *Macrosiphum gei*—were present at all centres, but the latter predominated during those seasons. Since, at this date, the specific nature of *Myzus persicae* as a transmitter of leaf-roll was not fully appreciated, no attempt was made to ascertain the precise numbers of each species. In subsequent observations each species has been recorded separately, and in the present paper the data regarding *M. persicae* have been extracted and compiled in Text-figs. 3, 4, 5 and 6.

(*a*) *Development of the aphis infestation.*

Over-wintering. In order to present a comprehensive story of the aphis infestation, it is proposed to summarise certain observations made on the hibernation, migration and distribution of *Myzus persicae*. (These aspects of the studies are being continued in detail.)

It was soon apparent that over-wintering eggs of *M. persicae* on peaches and nectarine (*Prunus persica*, Stokes var.) could account only for a mere fraction of the population of alate spring migrants that arrive on potatoes throughout the country during late May and June. Other sources of hibernation, such as plums (*Prunus domestica* (L.)), sloe (*P. spinosa* (L.)), hawthorn (*Crataegus* sp.), rose (*Rosa* sp.) were, therefore, examined for newly hatched nymphs, and later for alate *Myzus persicae*, but without result. In December, 1931, examination of a consignment of savoys (*Brassica oleracea* var.) at a large provincial market yielded apterous *Myzus persicae* in abundance on the under-surfaces of the leaves. Subsequent search among savoys under the severest weather conditions of the winters of 1931–3 proved that this mode of hibernation of *M. persicae* was general. During these winters, 100 per cent. of the plants examined at thirty-six different centres scattered throughout North Wales have been found to be harbouring colonies of apterous *M. persicae*. The savoy leaf, with its peculiarly wrinkled form, affords exceptionally favourable shelter for these hibernating aphides. It is essential that the host plant of hibernating apterae should not wilt during severe frosts, otherwise the aphides release their hold and fall off. Brassicae are thus very suitable. The savoy has the further distinct advantage in that, if weather conditions compel the aphides to release their hold, they are held within the small wrinkled compartments of the leaf until conditions are more favourable. Hibernating apterae of *M. persicae* have also been collected from other Brassicae, swedes (*Brassica Napus* (L.)), rape (*B. Rapa*) and other varieties of cabbage (*B. oleracea*), but the numbers during late winter have usually been much smaller than on savoys. The numbers of *Myzus persicae* found on Brassicae of various kinds in early spring, leaves no doubt concerning the *main* source of alate spring migrants. Since the population of *M. persicae* on potatoes will depend primarily on the number of alatae that arrive during the main migration in June, the proximity of a centre to areas where large quantities of winter Brassicae are grown will be a factor of some importance as a potential danger to a seed potato producing district.

Migration. The main migration of alatae takes place during late May and early June. With the exception of the dry season of 1933 when alatae arrived in quantity during late May and the first week in June, alate *M. persicae* have not been common on potatoes until mid-June.

During July, August and early September, alatae are by no means common on potatoes. These observations are supported by the data obtained from insect traps during these months. In late September and early October, the apterae can be seen developing wing buds and alatae become more common. The factors influencing migration are the subject of further study, but it will suffice here to state that there is definite evidence obtained from insect traps, that in North Wales the main migration of alate *M. persicae* is from the East. The greatest number of alatae arrive when the wind, or rather air—for the majority migrate when there is practically no wind—is from an easterly direction.

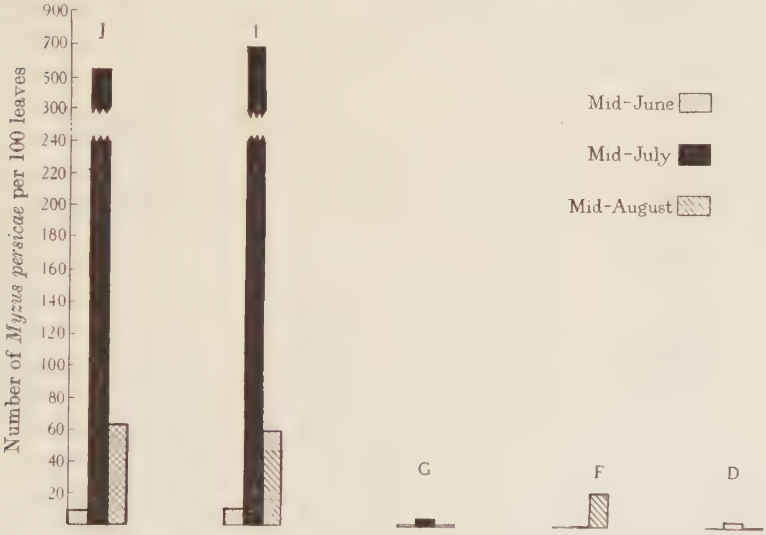
The main source and the migration of *M. persicae* having been indicated, it is now possible to proceed to a detailed study of aphis infestation at the different centres. The infestation of each year and at each centre is expressed in diagrammatic form in Text-fig. 3 (1930), Text-fig. 4 (1931), Text-fig. 5 (1932), Text-fig. 6 (1933).

(*b*) *Infestations of* Myzus persicae *at centres showing rapid increase of virus diseases in the potato stocks.*

Mid-June. At the mid-June inspection during 1928, 1929, 1930, 1931 and 1932 the infestation at the *unsuccessful* centres I and J (see Text-figs. 3–6) did not exceed 10 *M. persicae* per 100 leaves. Normally, then, the aphis infestation of potatoes has hardly commenced by mid-June; in fact, the alatae are still arriving at this date. This inspection, therefore, is too soon to give a correct indication of the degree of the aphis infestation at these centres.

The season 1933 was exceptionally dry and warm, as is indicated by the following meteorological data obtained at the College Farm, Aber, near Bangor. The total monthly rainfall (in inches) and the mean ($\frac{1}{2}$ (max. + min.)) monthly temperature for the period concerned was as follows: April, 0·67, 49° F.; May, 1·97, 53° F.; June, 1·90, 58·4° F.; July, 1·66, 62·1° F.; August, 1·65, 63·1° F. September, 1·46, 59·9° F. The weather conditions of April, May and June resulted in an exceptionally early arrival of alate *M. persicae* on the potatoes, so that as early as June 14th an infestation of 848 individuals per 100 leaves was recorded at centre J (Text-fig. 6), while at centre I it was of the order of 520 per 100 leaves. This was contrasted with the 10 aphides per 100 leaves recorded at this date in previous years. Obviously it indicated a phenomenal maximum infestation, but the dry conditions of July also favoured the reproduction of parasites and predators which markedly reduced the population of aphides. By mid-July (Text-fig. 6) the infestation had fallen to 158 *M. persicae* per 100 leaves at centre J and 120 per 100 leaves at centre I.

Mid-July. During 1928, 1929, 1930 and 1931 the maximum infestation at the unsuccessful centres (J and I) took place in mid-July. With the exception of 1931, this mid-July infestation was of the order of 600 *M. persicae* per 100 leaves. In 1931, with summer temperatures below normal, the maximum infestation was 118 per 100 leaves (see Text-fig. 4). In 1932 the first fortnight in June was cold, and during mid-July, also, the temperatures were below normal, with the result that development of the aphis population was delayed. In that season the maximum infestation was not reached until mid-August (see Text-fig. 5).



Text-fig. 3. Infestation of *Myzus persicae* in 1930. J, I. Unsuccessful seed potato producing centres. G, F, D. Successful seed potato producing centres.

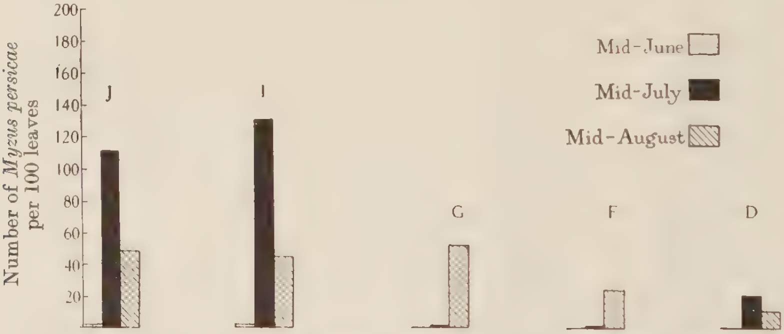
Mid-August. With the exception of 1932, the aphis infestation in mid-August showed a marked reduction (Text-figs. 3, 4 and 6), and did not exceed 62 *M. persicae* per 100 leaves during any of the other seasons. An inspection made during this period, therefore, would give a wrong conception of the nature of the aphis infestation which, normally, has declined by this date.

The exception took place in 1932 when, as stated, the weather conditions delayed the development of the infestation during June and July. The dry, warm days of early August, however, favoured a rapid increase and the maximum infestation reached the order of 1100

M. persicae per 100 leaves at centre J (Text-fig. 5) and 450 per 100 leaves at centre I.

(*c*) *Infestations of* Myzus persicae *at centres showing no increase of virus diseases in the potato stocks.*

Mid-June. The mid-June inspections of potato crops at the *successful* centres D, F and G (see Text-figs. 3, 4 and 5), with the exception of 1933, have not yielded a single specimen of *M. persicae*. This delay in the aphis infestation at the successful centres has been referred to previously (1). The exceptional year 1933 (see Text-fig. 6) affected the aphis infestation at these successful centres as at the unsuccessful ones, in that the initial infestation was much earlier. During mid-June it was of the order of 2, 10 and 23 *M. persicae* per 100 leaves at the centres G, F and D respectively.

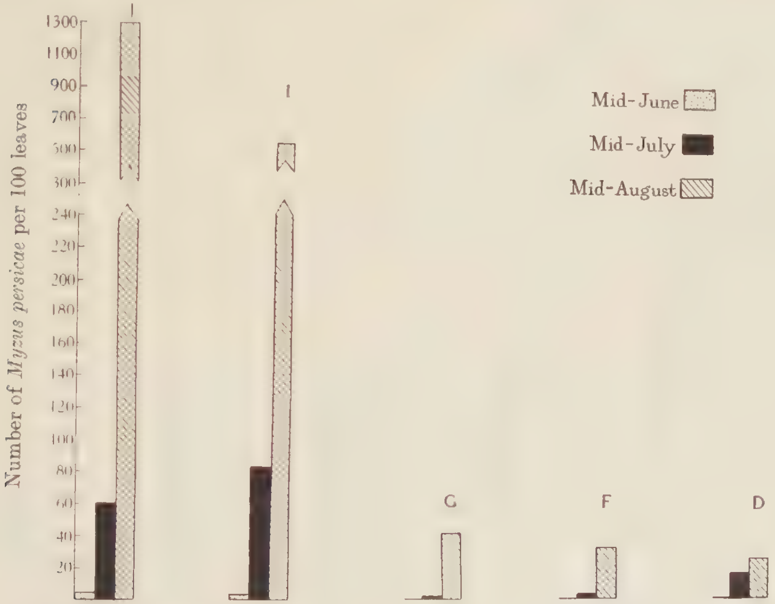


Text-fig. 4. Infestation of *Myzus persicae* in 1931. J, I. Unsuccessful seed potato producing centres. G, F, D. Successful seed potato producing centres.

Mid-July. At the mid-July examinations at centres F and G during 1928, 1929, 1930, 1931 and 1932 no infestation was recorded which exceeded 5 *M. persicae* per 100 leaves. At centre D, in Anglesey, the mid-July infestation has always been slightly higher than at F and G in South Caernarvonshire, but has not exceeded 20 per 100 leaves. The exceptional season of 1933 must again be dealt with separately. The earlier infestation of this year rapidly increased and at mid-July the infestation was of the order 82, 90 and 20 per 100 leaves at the centres G, F and D respectively. From observations of cast skins and parasitised aphides it was evident that at centre D the infestation had passed its maximum. Such a heavy infestation at these successful centres would be viewed with alarm unless it were realised that it indicates the degree of reproduction *within* the field and not the arrival of winged aphides from outside. This matter will be discussed later.

Mid-August. During the seasons 1928, 1929, 1930, 1931 and 1932 the aphis infestation in mid-August at centres G and F has shown an increase over that of the previous month. In 1931 and 1932 it exceeded 40 *M. persicae* per 100 leaves at centre G. The occurrence of the maximum infestation at these centres in mid-August is indicative of the delayed infestation and also represents the rate of reproduction within the crop.

The distinct fall in numbers (see Text-fig. 6) observed during mid-August 1933 is explained, as at the unsuccessful centres, by the activities of parasites and predators.



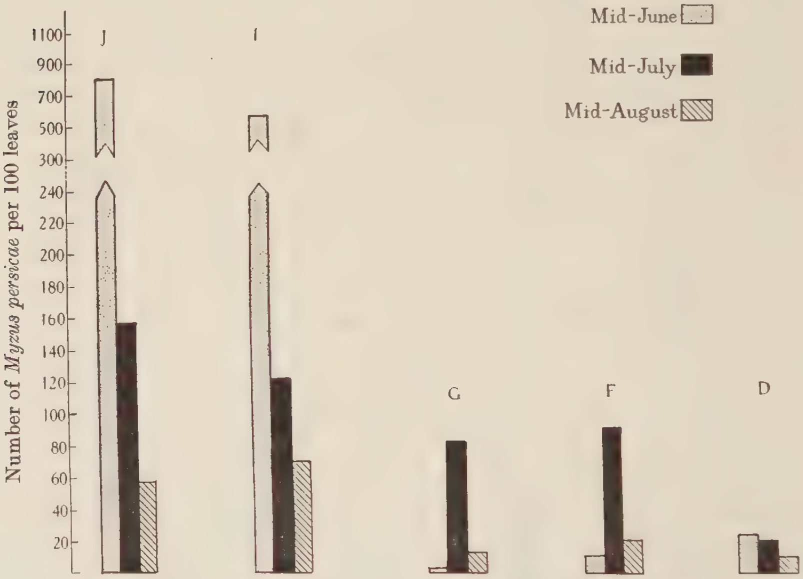
Text-fig. 5. Infestation of *Myzus persicae* in 1932. J, I. Unsuccessful seed potato producing centres. G, F, D. Successful seed potato producing centres.

It is important to note that at these centres in mid-August, and subsequently, the foliage of the potato crop rapidly matures.

(*d*) *Comparison of the total infestations of* Myzus persicae *at the successful and unsuccessful seed potato producing centres.*

1930. The *total* infestation of *M. persicae* at the *unsuccessful* centres I and J stands out in marked contrast to that of the *successful* centres G, F and D (see Text-fig. 3). At the former, the maximum exceeded 500 per 100 leaves and was reached during mid-July; at the latter (G, F and D) the maximum numbers did not exceed 20 per 100 leaves, and the period at which this low maximum was reached varied.

1931. In 1931 (see Text-fig. 4) the general infestation of aphides throughout the province was much lower than in the previous three years and, also, than in the subsequent two years. The maximum infestations at the *unsuccessful* centres were the lowest recorded during these observations. Even in this year, however, they were of the order 118 *M. persicae* per 100 leaves and 132 per 100 leaves at centres J and I respectively. The maximum at these centres was again reached during mid-July. At the *successful* centres during mid-July the infestation ranged as follows, 5, 5 and 18 per 100 leaves at centres G, F and D respectively. The maximum infestation, however, took place in August at centres G and F where it was of the order of 58 and 25 per 100 leaves respectively.



Text-fig. 6. Infestation of *Myzus persicae* in 1933. J, I. Unsuccessful seed potato producing centres. G, F, D. Successful seed potato producing centres.

1932. Season 1932 (Text-fig. 5) yielded the highest numbers of *M. persicae* recorded during the years in which observations were made. The maximum infestations at the *unsuccessful* centres J and I were 1103 per 100 leaves at centre J and 525 per 100 leaves at centre I. As stated, the period of maximum infestation was delayed until mid-August. During mid-July, however, it was quite appreciable, being 60 and 83 per 100 leaves at centres J and I respectively. At the *successful* centres

G, F and D the maximum infestation, also, took place in mid-August but was only of the order of 44, 35 and 28 per 100 leaves at the respective centres. It was clear, also, that this infestation had been reached owing to rapid reproduction within the field since mid-July. At the latter date the infestation was only 4, 5 and 20 individuals per 100 leaves respectively at the centres G, F and D.

1933. The exceptional conditions of this season have been noted, and although they affected the nature of the infestation they did not alter the contrast in the magnitude of the infestation at the different types of centres. The maximum infestation at the *unsuccessful* centres was of the order of 848 *M. persicae* per 100 leaves at centre J and 520 per 100 leaves at centre I. This occurred during mid-June. The delay in infestation at the *successful* centres was again evident and the maximum was not attained until mid-July. This was the highest infestation recorded at the successful centres reaching 82 *M. persicae* per 100 leaves at centre G and 90 per 100 leaves at centre F. At centre D during mid-August the infestation was 20 per 100 leaves but there was evidence that this was a post-maximum figure.

V. Discussion.

In addition to advancing knowledge of the nature of the aphis infestation of the potato crop, the aim of the survey was to ascertain if an *index* figure could be obtained which would be indicative of the degree of aphis infestation that had limited the successful production of seed potatoes owing to the rapid introduction and spread of virus diseases among the stocks. At the unsuccessful centres (I and J) where the spread of virus diseases has been rapid, the *total* infestation of *Myzus persicae* has always far exceeded that of the successful centres (D, F and G), where there has been no increase in virus infection. The maximum infestation at the unsuccessful centres has always exceeded 100 *M. persicae* per 100 leaves, and in three seasons out of four has exceeded 500 individuals on the same leaf area. At the successful centres, on the other hand, the maximum infestation has never attained 100 per 100 leaves. It is important, however, to realise that a high maximum infestation need not be indicative of a rapid spread of virus diseases, since it may represent only the amount of reproduction of aphides that has occurred *within* the crop. If the crop is originally healthy, a rapid increase of apterous aphides within the field would not be followed by a spread of virus diseases. Thus, at centres D, F and G, the aphis infestation during August has reached such figures as 40, 50 and 80 *M. persicae* per 100 leaves without resulting in any increase in virus infection, simply because the figures during August indicate the rate of reproduction of the aphides within the crop and not the intensity of the initial infestation of alatae from outside sources. The figures of the infestation in mid-August, then, would be of little value as an indication of the increase in virus diseases to be expected. Clearly, it is the numbers of alate *M. persicae* that arrive from *without* the crop, and the degree to which these spread from plant to plant, that constitute a potential danger to a seed potato producing centre. It is the figure that supplies this information that is required as an index.

It has been explained that, normally, the main migration of alatae takes place in early June, and that these insects will continue to move from plant to plant for about a month. By early July the migration will have ceased. Thus, in an average season, the mid-July infestation can be regarded as the best indication of the information required. The condition of growth of the potatoes at this period is shown in Plate XVI, fig. 2 (Kerr's Pink) and Plate XVI, fig. 1 (Great Scot). It is necessary, however, that this index figure should be reliable in all types of seasons. In a season such as 1932 (Text-fig. 5) when the initial arrival of alatae is delayed, owing to unfavourable weather conditions, the mid-July figure will still be indicative of the intensity of the migration, since in the latest seasons the main migration of alatae will have been completed by early July. Modification is necessary, however, in a season such as 1933 (Text-fig. 6), when the arrival of alatae was exceptionally early and intense. The mid-July figure for the aphis population in such a season may be misleading, since the infestation may have already reached its maximum and have been reduced by the activities of predators and parasites. In the field, such a condition can be detected owing to the sticky nature of the honey-dew covered leaves, the presence of a large number of white cast-skins, and a high proportion of parasitised aphides, all pointing to a previous heavy infestation. In a season such as 1933, therefore, when late May and early June are particularly warm and dry, it will be necessary to accept the mid-July index figure with certain reservations. Either it can be regarded as a minimum index figure, or else, in such a season, an additional examination should be carried out during mid-June. The remaining alternative would be to regard the mid-June infestation as the index figure for the aphis population at a given centre but, unfortunately, this is unsatisfactory, for in most seasons the main migration of alatae has either not taken place or is in progress at this date.

It is now possible to discuss the order of magnitude of the index figure that will be indicative of a degree of aphis infestation sufficient to prevent the successful production of seed potatoes owing to the rapid introduction and spread of virus diseases among the stocks. From the data given in Text-figs. 3, 4 and 5 it will be seen that, excluding the exceptional year 1933 when the development of the aphis infestation was almost a month in advance of previous years, the mid-July infestation at centres D, F and G, at which there has been no increase in virus infection, has not exceeded 20 *M. persicae* per 100 leaves. From these and similar observations at the other fifteen successful seed producing centres in the scheme, it is evident that if the infestation in mid-July does not exceed 20 *M. persicae* per 100 leaves, there is every prospect that the centre will prove successful, because the virus infection that is spread into the original healthy stock will be negligible. On the other hand, if the mid-July infestation exceeds 100 *M. persicae* per 100 leaves, and, certainly, if it exceeds 500 individuals per 100 leaves, the centre is likely to prove unsatisfactory for the production of seed potatoes owing to the rapid increase in virus infection that is probable among the stock. Even if, during a particular season, the potatoes in adjacent districts do not include a high percentage of virus infected plants, such an aphis infestation is always a potential danger to the seed producing centre. If the mid-July infestation gives an index figure between 20 and 100 *M. persicae* per 100 leaves, the conclusions would be uncertain, unless there was evidence, from the prevalence of cast skins and parasites, that the maximum infestation had already taken place and that the mid-July figure therefore represented a post-maximum index figure. In the latter case the centre could be discarded. If the maximum infestation has not taken place, as indicated by the absence of cast-skins and parasites, then an index figure of 20–100 *M. persicae* per 100 leaves would be either the absolute maximum, which in an early season would be permissible, without expectation of an increase in virus infection; or, the figure would be the sub-maximum, which could be checked by a count taken in mid-August, when, if there was any increase in the aphis infestation, the centre could be discarded.

In view of the general mode of hibernation of *M. persicae* as apterae on Brassicae, particularly savoys, the proximity of a centre to districts where large quantities of winter Brassicae are grown will have an important bearing on the intensity of the population of *M. persicae* on the subsequent potato crop. The centres which have been discarded from the scheme, owing to the rapid increase in virus infection are nearer to market gardening and horticultural areas, and, while this is not believed to be the sole factor accounting for the difference in intensity of the aphis population at the respective centres, it undoubtedly plays a major part. In practice, also, areas in which a large quantity of Brassicae are grown are those in which potatoes also are largely grown, so that the possibilities of the aphides becoming vectors of virus diseases is increased.

VI. SUMMARY.

1. A comparative study has been made during 1928–1933 of the aphis infestation at *successful* seed potato producing centres, where there has been no increase in virus infection among the stocks, and also at *unsuccessful* centres, where the increase in virus infection has been large and rapid.

2. The technique developed for this study—one which is applicable for an aphis survey of prospective seed potato producing areas—is given. Identification, in the field, of the different species of aphides infesting potato is desirable in such a survey, and descriptions, with photographs, are presented.

3. Ecological notes on the development of the aphis infestation show that *M. persicae*—the species that is generally responsible for the spread of virus diseases among potatoes—hibernates as apterae in considerable quantity on winter Brassicae, particularly savoys. These plants constitute the main source of alate spring migrants in most rural districts. The main migration of alatae takes place, normally, in early June, and, in North Wales, is largely from the east. Alatae are not common during July and August and do not increase until the late September migration from potatoes.

4. The index figure of infestation at centres in which there was a rapid increase in virus diseases among the potato stocks always exceeded 100 *M. persicae* per 100 leaves, and attained such numbers as 1000 individuals per 100 leaves. At centres where there has been no increase in virus infection, the index figure of infestation has not exceeded 20 *M. persicae* per 100 leaves. The period at which this index figure should be obtained is important, and, with certain exceptions, mid-July proved to be the most satisfactory. It is essential that the figure should be an index of the intensity of the initial infestation of alatae from *outside* sources and their subsequent spread, and should not be simply an index of the rate of reproduction of aphides *within* the field.

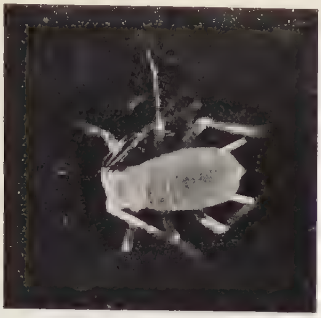
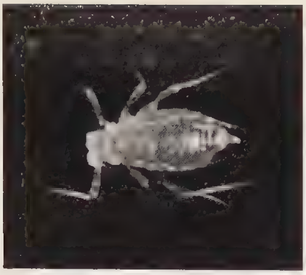


Fig. 3.



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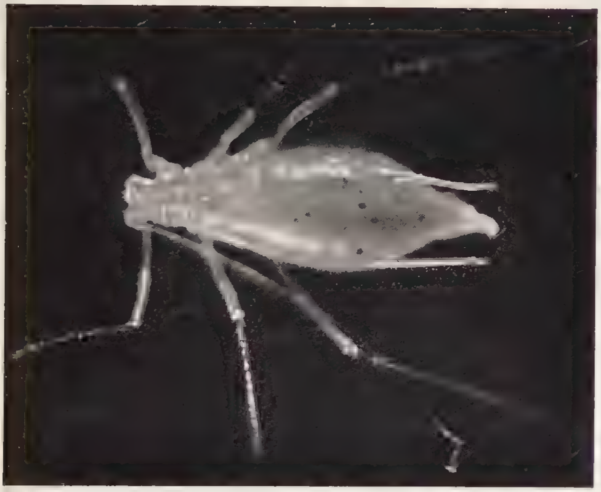
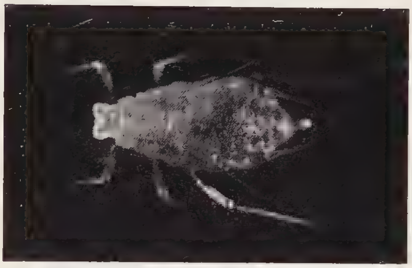


Fig. 2.



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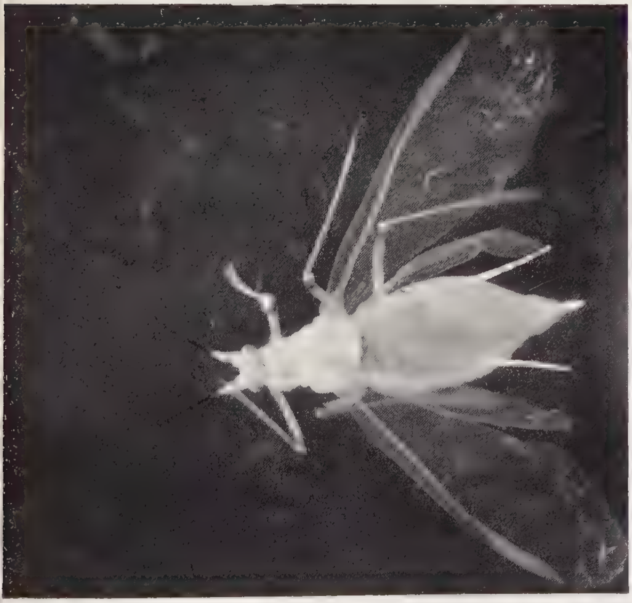

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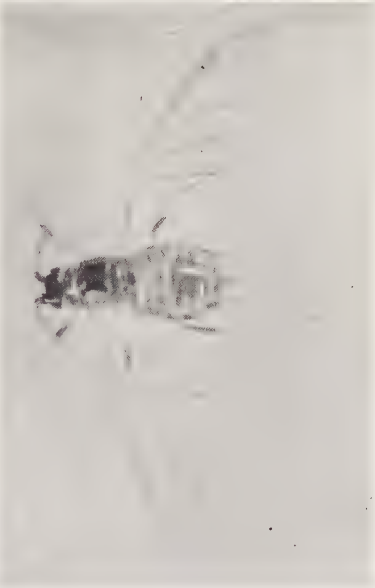

Fig. 4.

DAVIES.—Studies on Aphides Infesting the Potato Crop (pp. 283–299).

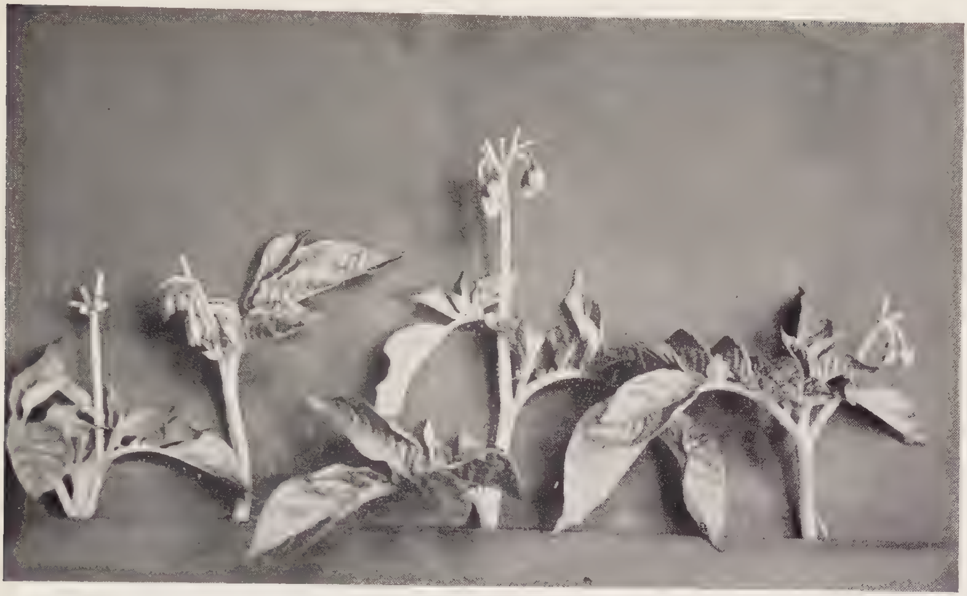

Fig. 1.

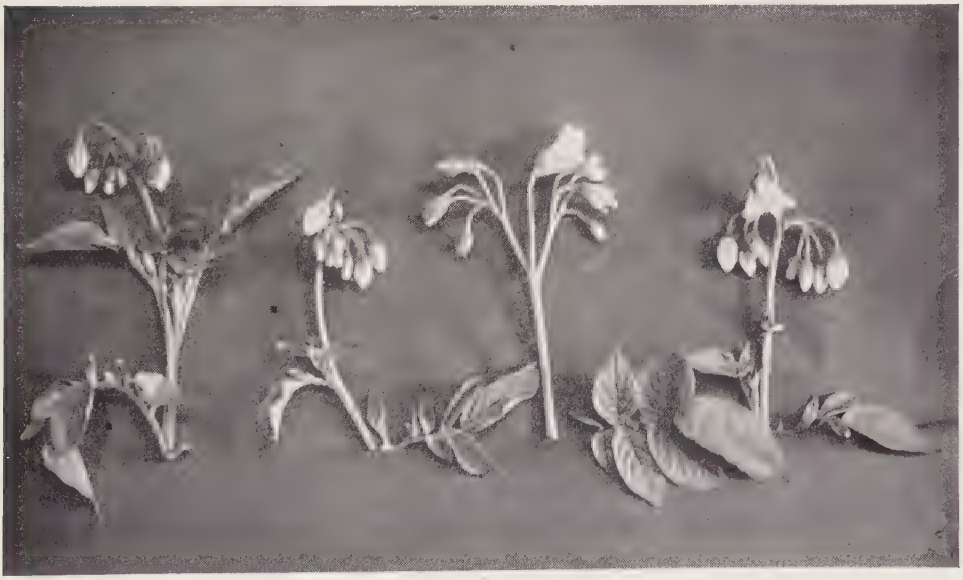

Fig. 2.

DAVIES.—Studies on Aphides Infesting the Potato Crop (pp. 283–299).

The writer wishes to express gratitude to Mr J. C. F. Fryer and to Professor R. G. White for their helpful suggestions during the progress of the work and, also, for their criticism of the manuscript.

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EXPLANATION OF PLATES XV AND XVI.

PLATE XV.

Fig. 1. Alate *Macrosiphum gei* Koch. × 10.
Fig. 2. Apterous adult *Macrosiphum gei* Koch. × 12.
Fig. 3. Nymph (1 day old) *Macrosiphum gei* Koch. × 12.
Fig. 4. Alate *Myzus persicae* Sulz. × 12.
Fig. 5. Apterous adult *Myzus persicae* Sulz. × 12.
Fig. 6. Nymph (1 day old) *Myzus persicae* Sulz. × 12.

PLATE XVI.

Fig. 1. Condition of growth of the variety "Great Scot" at the time of mid-July inspection.
Fig. 2. Condition of growth of the variety "Kerr's Pink" at the time of the mid-July inspection.

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ON THE PIGMY MANGOLD BEETLE (*ATOMARIA LINEARIS* STEPH.) AND METHODS FOR ITS CONTROL

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(With Plates XVII and XVIII.)

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I. ECONOMIC IMPORTANCE.

THERE is perhaps none of the common insect pests of the sugar beet and mangold crops that more urgently needs attention at the present time than the Pigmy Mangold Beetle (*Atomaria linearis* Steph.). In the years since the War, particularly since the advent of sugar-beet growing, complaints of its increasing prevalence have been made from all parts of England. The losses inflicted by this beetle in many counties are believed to be greater than those caused by any other pest incident during the seedling stage of the mangold and the sugar beet. Where a severe outbreak occurs it is by no means uncommon for 70 per cent. of the plants to die, and for the remainder to be so stunted in growth that their market value is greatly reduced. The magnitude of the financial loss directly caused by this insect is not generally realised, for the damage is often erroneously attributed to other causes, such as inclement weather, faulty germination, wireworm attack, manurial deficiency, or unsuitable soil conditions.

II. DISTRIBUTION.

This beetle occurs throughout the greater part of Europe and is regarded in many countries as a serious menace to the sugar-beet industry. In Great Britain severe attacks have been reported (7) from Cornwall, Devon, Somerset, Dorset, Wiltshire, Hampshire, Sussex, Kent, Buckinghamshire, Oxford, Northamptonshire, Huntingdonshire, Cambridge, Suffolk, Norfolk, Lincolnshire, Nottinghamshire, Derbyshire, Yorkshire and Shropshire.

III. HISTORICAL ACCOUNT.

The publications on the Pigmy Mangold Beetle, prior to the year 1932, have been summarised by Newton (8), so that detailed consideration of this earlier work is not necessary, but references to the most important of these papers are included in the list of references (1–6, 9–11). The insect has been much neglected in the past by British entomologists, and in spite of its long-continued and injurious depredations no detailed account of its life history and habits exists, and the problem of its control has never been the subject of scientific investigation.

In view of these circumstances, the senior author (E.E.E.) decided in 1927 to make an attempt to supply some of the missing data and more especially those necessary to the solution of the economic problem raised.

The present paper embodies (*a*) general field observations of infested areas, both in Lincolnshire and Shropshire, over a period of six years, and (*b*) results obtained in 1928 and 1929 from field experiments on the control of the beetle. Delay has been inevitable in the preparation of this paper, partly because one of the authors was appointed to a post in a part of the country remote from where the trials were started, and partly because it was considered necessary to obtain further and more conclusive evidence of the value of the results of the 1928 and 1929 field experiments.

IV. Description and life history.

The beetle is considerably less than a tenth of an inch in length, very narrow, parallel sided, and of a dingy reddish brown colour. The beetles immediately drop and feign death when disturbed. This habit, in conjunction with their small size and colour, probably accounts for the past failure of growers generally to associate them with the particular form of damage for which they are responsible. From the beginning of May till about the middle of July the beetles may be seen in large numbers feeding on the roots and foliage of mangold and sugar beet. Presumably the eggs are deposited in the soil near the plants attacked. The larva lives in the soil adjacent to the root system of the plants for a period of about six weeks. It is elongated, grey in colour and with three pairs of weakly developed thoracic legs, and when fully grown measures approximately 1/10 in. in length. Pupation takes place in early August, at least under laboratory conditions. The length of time in the pupal stage is not known, and all attempts by the writers to determine the length of the pupal period have been abortive. Immature beetles, however, have been noted in the field during the latter part of September. These beetles hibernate in the soil or among debris.

V. Symptoms of attack in mangold and sugar-beet crops.

The earliest visible symptom of attack is poor germination, since the adult beetles feed on the shoot of the seed as it germinates; severe infestation may result in complete failure of the crop. Attacked seedlings can be easily recognised, in the first instance, by their poor growth, and, subsequently, by the yellowing and wilting of the foliage. If such seedlings are gently pulled they will be found to come up very readily, and closer examination will reveal that the root system and the lower part of the stem have been badly attacked. In most cases the beetles themselves will be found in large numbers under clods of earth and particularly in the soil around the roots. The injury to the root system consists of the destruction of many or all of the finer roots and of small excavations eaten out from the main root (Plate XVII, fig. 1). In severe attacks, a black constriction just below ground level is generally apparent, and often the young infested root presents a characteristically threadlike appearance as shown in Plate XVII, figs. 1 and 2, respectively.

The beetles attack not only the roots but also the foliage. At first, small irregular holes somewhat resembling damage by flea beetles (*Phyllotreta* sp.) are eaten in the leaves, but this kind of injury is usually small and unimportant. Later, the crown or growing point of the plant is attacked and instead of normal leaves, a number of stunted, deformed leaves are produced (Plate XVII, fig. 3). In severe infestation the growth of the plants is badly retarded and sometimes may even be completely checked. The intensity of this kind of attack, however, is markedly influenced by the nature of the soil and the weather conditions prevailing during the seedling period. The most serious injury to the foliage is caused in cold damp weather and on the heavier types of soil, whilst the least amount of leaf damage occurs in the drier seasons and on the lighter types of soils.

VI. CIRCUMSTANCES ATTENDING OUTBREAKS OF THE BEETLE.

The general field observations, made by the writers both in Lincolnshire and Shropshire during the past six years, indicate that the Pigmy Mangold Beetle, though often present in small numbers on sugar-beet and mangold crops, only assumes an epidemic form under certain conditions. The most serious outbreaks are usually found in those districts where repeated cropping of the land with sugar beet or mangolds has been practised over a number of years. Once the pest becomes well established, the increase in numbers is so rapid that successive cropping of the infested land, especially by sugar beet, usually has to be discontinued. An epidemic does not, however, always follow continuous cropping of sugar beet or mangold, as several instances of infested areas are known to the writers where these crops have been successfully grown for many years in succession without any apparent increase in the beetle population. It would, therefore, appear that some factor or a combination of factors other than continuous cropping is essential for an unusual development of this beetle in areas devoted to sugar beet or mangold.

VII. THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON DEGREE OF INFESTATION.

(1) *Rotation.*

A suitable rotation of crops is an important factor affecting infestation by the Pigmy Mangold Beetle. It is found that a rotation whereby susceptible crops are not taken too frequently on the same land has a very marked beneficial influence as shown by the following examples. In Field A in 1927 the sugar-beet crop was extremely poor owing to an attack by the Pigmy Mangold Beetle. A rotation of crops was then taken, cereal in 1928, clover and seeds mixture in 1929, potato in 1930. The field thus rested for three years rewarded the farmer in 1931 with a very good crop of sugar beet. Another example was provided by Field B where the mangold crop in 1928 suffered severely from Pigmy Mangold Beetle. After four years, during which neither mangold nor sugar-beet crops were grown, sugar beet was sown in 1932. An excellent crop was lifted, yielding an average of 14½ tons of washed beet to the acre. It would appear then, that if land infested by this pest is rested for a number of years from sugar beet and mangolds, other crops being substituted, a satisfactory crop of either sugar beet or mangold can then be grown. The exact length of time that should intervene between one crop of sugar beet or mangold and the next has not yet been definitely ascertained. Instances are known where satisfactory sugar-beet crops have been obtained after an interval of one year, but a few others proved almost complete failures after such a short rest. It is possible that the conditions associated with the growing of sugar-beet crops may account for these contradictory observations, as some evidence was obtained in a field in South Shropshire in 1932 that a satisfactory crop can be grown after a rest for one year provided that the debris from the sugar beet, in the form of crowns, bolters, etc., is carted off the land or carefully folded off by sheep, and not left in the field. The crop in this field was badly attacked in 1930 and produced only about 5 tons of washed beet to the acre. The debris from the crop on one part of the field was partly carted off and partly folded off by sheep (separate areas), whilst that on the other part was left in the field. Subsequent treatment was similar for both parts. The whole field was potatoes in 1931 and again sugar beet the following year. In 1932 the sugar beet on those parts of the field where the debris had been carted or folded off in 1930 was an excellent crop, while the beet on the part where the debris had been left on the land in 1930 suffered from attacks by the Pigmy Mangold Beetle and yielded on an average only 7½ tons to the acre.

It must not be inferred that control of the pest can always be obtained on infested land, even by means of rotation whereby successive crops of sugar beet or mangold are not taken more often than once in three years, as two instances have been noted by the writers of attacks after an interval of three and five years, respectively. It was found, however, in the former case that the weed—Goosefoot or Fat Hen (*Chenopodium album*)—was very abundant in the field and heavily attacked by the Pigmy Mangold Beetle, and it is possible that this weed provided suitable food in the interval for the beetle; in the latter case, no explanation was found.

(2) *Soil factors.*

No evidence was obtained in the course of the field observations made, that the Pigmy Mangold Beetle is restricted to any particular type of soil. Generally speaking, plants grown on soils which are of a sandy character and in a low state of fertility usually suffer more from damage to the germinating shoot and to the root system than those growing on heavier and more fertile soils, at least, in a normal growing season. Some correlation appears to exist between a low potash content of the soil and outbreaks of attack.

(3) *Manurial treatment.*

Observations made on infested fields which had received different manurial treatment in various districts in both Shropshire and Lincolnshire have revealed that the intensity of infestation varies widely with the kind of manurial treatment adopted. The most probable explanation of this fact is found in the differences exhibited on different plots in the rate of early growth of the plant. Any manures which stimulate the plant so as to hasten it through the seedling stage are likely to have a beneficial effect in reducing the extent of the damage. On most soils, with the exception of the fen soils in Lincolnshire, it was found that the use of increasing quantities of nitrogenous fertilisers in conjunction either with farmyard manure or complete minerals consistently brought about a reduction in the amount of injury; on some of the fen soils, however, heavy applications of potash proved most beneficial in this respect.

(4) *Climatic factors.*

Intensity of attack by this beetle may vary from season to season according to the climatic conditions prevailing. The factors chiefly contributing to an epidemic attack are low temperature and a very dry or very moist atmosphere during the seedling period. These conditions retard the development of the seedlings, and cause them to remain susceptible to attack for a longer period than usual.

Climatic conditions during the early stages of growth of the plant have also an important bearing on the nature of the damage done by this beetle. In a normal season the greatest injury is suffered, in the first instance, by the shoot of the seed as it germinates and later by the root system of the plant. Foliage damage is almost negligible. Abnormally damp weather, on the other hand, results, particularly on heavier types of soil, in an appreciable amount of foliage damage and correspondingly less injury to other parts of the plant. Any climatic factors that prolong the period between the time the seed germinates and the appearance of the seedlings above ground have a considerable influence on the amount of damage to the shoots. Thus, heavy rains followed by a very rapid dry period shortly after the seed is sown may cause the formation of a hard crust on some soils which hinders the normal rate of penetration of young seedlings through the ground and, under these conditions, complete failure of crops on infested land due to shoot injury is of common occurrence. It is believed that the responsibility for this type of damage may be fairly laid to the charge of the Pigmy Mangold Beetle in a great number of cases formerly attributed to very different causes.

(5) *Soil consolidation.*

The degree of soil consolidation has considerable influence upon the susceptibility of plants to attack. Plants grown in a firm seed bed suffer less than those growing where consolidation of the soil has been neglected. The great value of using the roller was well exemplified in a field of sugar beet on a farm at Eaton Constantine, Shropshire in 1931. The field was 12 acres in extent. Half of it was rolled twice immediately after sowing and again at weekly intervals until the plants were well established, while the other half was used as control. The previous cropping, cultivation and manuring of both parts of the field had been the same. The effect of rolling was most marked both on the plant and the intensity of attack by the pest. Quicker germination and a more rapid rate of growth throughout the period subject to beetle injury was noted. The extent of damage in the germinating stage was somewhat less as shown by better germination of the seed, owing probably to the shorter period between the time the seed germinated and the appearance of the seedlings above ground. The reduced root injury observed can possibly be explained by the enhanced speed of the plants' growth or by the inability of the beetles to burrow freely from plant to plant in a closely packed soil. The increase in yield as the result of the rolling was at the rate of approximately 2 tons of washed beet to the acre. The infestation was not, however, very severe in this case, as the "unrolled" portion of the field produced 9½ tons to the acre. Several other instances were observed where soil consolidation had advantageously affected the crop, but it is not considered that thorough compression of the soil alone is sufficient to control the pest except perhaps in areas which are but slightly infested.

(6) *Extra seeding.*

Perhaps one of the most important points in successful sugar-beet or mangold husbandry is thick sowing of the seed, and many farmers have yet to learn its supreme importance, particularly in areas subject to depredation by insects and other pests. Several instances were noted which appear to justify extra seeding as a precaution against loss of crop from the Pigmy Mangold Beetle, and it is interesting that Miss Ormerod (9) as far back as 1895 suggested thick seeding as a method of control. The method is practised at the present time with some measures of success in districts on the Continent where this beetle is a serious pest to sugar beet.

In order to investigate the possibilities of using this method a series of preliminary field experiments was laid down in 1929 in Lincolnshire and Shropshire on areas that had suffered from attacks by the Pigmy Mangold Beetle during the previous season. At each centre plots were drilled at the rate of 15, 25 and 35 lb. to the acre. These experiments showed that increased quantity of seed, though somewhat effective as judged by the increased yield of crop in instances of slight infestation, had little or no influence in cases of heavier infestation.

(7) *Time of sowing.*

In 1928 an exceptionally severe outbreak of Pigmy Mangold Beetle occurred in a field of sugar beet on a farm at Holbeach, Lincolnshire. Half of the field had been sown four weeks earlier than the other half, the respective dates being April 17th and May 8th. The earlier sown part of the field was so badly attacked that it had to be ploughed up, while the later sown showed considerably less damage and produced a fairly satisfactory crop. The whole field was uniform as regards previous cropping, cultivation and manuring for the sugar-beet crop. On the other hand, other instances have been noted where sugar beet sown earlier was less severely attacked than similar late-sown seed.

(8) *Time of thinning.*

In Denmark, late thinning out of the plants is advised in areas liable to infestation, and some evidence confirming the value of this advice was obtained in the present investigation. It was found that the severity of attack may be reduced to some extent by late thinning when the plants are 3–3½ in. high as compared with thinning when the plants are but 1–1½ in. in height. The degree of control given is small, but this practice is considered worthy of adoption in cases of slight infestation. Late thinning, however, often cannot be accomplished to advantage, particularly in heavy cold soils, but the thinning should take place at the latest possible date with due reference to climatic and soil conditions.

VIII. VARIETAL SUSCEPTIBILITY OF SUGAR BEET AND OTHER CROPS TO ATTACK.

Different varieties of sugar beet vary in the degree to which they are liable to infestation, but the differences are generally small and unimportant, probably because the varieties are so closely related. The infestation figures obtained in the course of this investigation were not constant, since facts collected from one affected field were negatived by observations in the next or even on the same field in another season. It was evident that the variation in susceptibility to injury shown by different varieties does not imply that the beetles themselves prefer one variety to another, but rather that in certain varieties the powers of recovery are much greater. Such powers of recovery appear to be dependent not only on the constitution of the variety but also on the season and suitability of the soil for the variety concerned.

Mangolds as a rule are more resistant, and have been observed, comparatively free from attack, growing side by side with infested sugar beet. Beetroots do not appear to suffer as a rule, but a single instance of slight attack on this crop was noted during the past season in a field at Wellington, Shropshire. According to Lagenbuch and Schewket (6) who have studied the beetle in Germany, spinach, radish and marjoram are also subject to attack.

IX. SOURCES OF INFESTATION.

From certain field observations made it would appear that the debris of attacked sugar-beet crops, in the form of crowns and bolters, may possibly act as sources of infestation. Some correlation has been noted on occasions between outbreak of the beetle and the deposition of such debris on the field, for feeding purposes, during the previous season. Odd pieces of crowns and bolters from the previous crops have frequently been found in the autumn and, on two occasions in the late spring, to be swarming with the beetles which were either concealed in cuts and cracks or feeding on the new leaves that were being thrown out from the old crowns. Reference has already been made to a possible correlation between the failure of two-year course rotations to keep the beetle under control on infested land and the practice of leaving the debris to rot on the ground.

Observations made on an outbreak in the Lane Field at the Kirton Agricultural Institute Farm in 1929 strongly suggested that the growing of sugar-beet crops in close proximity to infested areas should not be practised. The field had not previously grown a crop of either sugar beet or mangold. The most serious damage occurred where there is no definite boundary between this field and the Aspland field which was planted with potatoes but in 1928 had grown a sugar-beet crop that suffered severely from an attack of the beetle. The photographs reproduced in Plate XVIII, figs. 1 and 2, give some idea of the difference in the intensity of attack in the Lane Field in 1929. Fig. 1 shows the appearance of the crop near the Aspland boundary where the number of plants had been appreciably reduced and Fig. 2 shows the crop nearer the centre of the field where the damage was negligible from a practical standpoint. Between these two extremes no distinct line of demarcation was apparent but rather a progressive graduation of one into the other.

Further, Goosefoot or Fat Hen (*Chenopodium album*), an all too common weed in sugar-beet and mangold crops in many localities, is occasionally attacked, and consequently special care should be taken to keep it down, particularly in those infested areas where a rotation of crops is the rule.

X. FIELD CONTROL TRIALS.

(1) *Field experiments*, 1928 *and* 1929.

With a view to testing certain soil treatments and seed dressings under the conditions usually prevalent in Holland Division of Lincolnshire, preliminary experiments were laid down on the Institute Farm at Kirton in 1928. These were sufficiently promising to make a second trial advisable, and the results obtained in 1929 are discussed fully, since these experiments were the more comprehensive and are typical of both years.

A. *Experimental treatments employed.*

Four soil treatments and three seed dressings were tested in these trials.

(i) *Soil treatments.* The following four substances were applied to the respective plots at the rates given below:

(*a* Whizzed naphthalene at the rate of 3 cwt. per acre.
(*b*) "Drained creosote salts" at the rate of 3 cwt. per acre.
(*c*) Calcium cyanamide at the rate of 6 cwt. per acre.
(*d*) Calcium cyanide at the rate of $5\frac{3}{4}$ cwt. per acre.

All these chemicals were applied fourteen days before the seed was sown. They were broadcast and ploughed in immediately after application to a depth of about 7 in. All the plots were then given a double harrowing, followed by rolling with a heavy roller, in order to impregnate the soil thoroughly with the chemicals and to avoid as far as possible the escape of their vapours.

(ii) *Seed treatments.* The following three seed dressings were employed:

(*a*) *Magnesium sulphate and phenol* (12). Seed was immersed for 20 min. prior to sowing in a solution of 5 parts magnesium sulphate and 1 part phenol in 100 parts of water. This seed was then drained and sown with as little delay as possible.

(*b*) *Corrosive sublimate* (*mercuric chloride*). Seed was steeped for an hour before sowing in a solution of 1 part corrosive sublimate in 1000 parts of water (1 oz. in 6¼ gal.).

(*c*) *Naphthalene.* Grade 16 naphthalene was mixed with the seed. This was used at the rate of 1 part by weight to 20 parts of seed.

B. *Plan of the experiment.*

The variety of sugar beet used for these experiments was Perfection, an English-grown seed. The experimental area was divided into eighteen plots of area 1/40 acre, each of which contained eighteen rows of sugar beet and was separated from its neighbour by a path 2 ft. wide. The distance between the rows was 18 in. and a singling distance of 9 in. in the row was aimed at. The seed was sown on April 29th at the rate of about 25 lb. to the acre. The whole area was uniform as regards previous cropping, cultivation and manuring. The previous cropping was—1925, oats; 1926, mangolds; 1927, sugar beet; 1928, sugar beet. About 15 tons of farmyard manure per acre were applied in February, 1926, and ploughed in with the oat stubble; 2 cwt. superphosphate, 1½ cwt. muriate of potash and 2 cwt. nitrate of soda were applied at the time of sowing of sugar beet in 1927 and 1928.

The seven different treatments were duplicated so that fourteen plots were used, while four untreated plots served as controls.

C. *Effect of treatments on seed germination.*

The effect of the various treatments on the germination of the seed was very marked as judged from the seedlings at the time of their appearance through the soil. The seedlings on the plots sown with seed treated with the phenol-magnesium-sulphate solution appeared above ground some 4 or 5 days earlier than those on the control plots. This stimulating effect on the seed was also accompanied by satisfactory germination or number of seedlings and more uniformity in the rate of growth as compared with the control plots which were obviously seriously affected by the great prevalence of Pigmy Mangold Beetle. The seedlings on the plots sown with naphthalene-treated seed, on the other hand, were less numerous and decidedly slower in appearing through the ground than those on the controls. This may be attributed to an adverse effect of the treatment on germination and a retardation of the appearance of the young plants above ground, thus lengthening the critical period when the seed is liable to "shoot attack" by the beetle. It has since been found by the writers in several other fixed trials that naphthalene, at least "Grade 16," when mixed with the seed in the ratio 1:20 greatly impairs the germination of sugar beet. The seedlings on the remaining plots were not significantly affected adversely or advantageously by any of the other treatments.

D. *Effect of treatments on growth and vigour of the plants.*

A decided difference in the growth and vigour of the seedlings could be detected between the various plots and as the season progressed the differences became more and more marked and, by the beginning of June, were most striking. At this time the plants on the plots sown with seed treated with phenol-magnesium-sulphate solution were very even in size, growing vigorously and on the whole of a healthy colour. They were 6 or 7 days more advanced in growth than those on the control plots. This difference was maintained for some weeks, but ultimately the plots were indistinguishable as regards growth. The untreated or control plots contained many unhealthy plants due to an attack of Pigmy Mangold Beetle and the majority were much stunted; the plants were also very irregular in size and making little growth. The calcium cyanamide plots showed but little improvement over the controls. The remaining plots were about equal, being badly attacked and comparable in appearance with the controls.

E. *Effect of treatments on the incidence of Pigmy Mangold Beetle.*

Examination of the plots soon after germination of the seed revealed that beetles were present on both treated and control plots. Severe damage by the beetles to the shoots of the seeds as they germinated was apparent on all plots except on those sown with seed treated with the phenol-magnesium-sulphate solution. It was calculated that on an average approximately 30 per cent. of the seedlings had been destroyed in this way on the former as compared with about 12 per cent. on the latter. This difference in the number of seedlings destroyed is believed to be due, at least to a large extent, to a stimulating effect of the phenol-magnesium-sulphate treatment on the growth of seedlings in the germinative stage, thus shortening the critical period when the sugar-beet plant is liable to attack. Laboratory observations indicate that this treatment also renders some direct protection to seedlings in the early stages of their development, but conclusive experimental evidence on this question is not yet available.

As the season advanced, serious attack by the beetles on the root system of the young seedlings became evident. The plots sown with seed treated with phenol-magnesium-sulphate solution again suffered appreciably less injury than any of the others. The system of marking employed for assessing the amount of injury was to judge each plot as a whole, 10 marks being allotted where the number of plants destroyed was severe, with graduations down to 0 where the damage was negligible from the practical standpoint. An average of 3 marks was recorded in this way on the phenol-magnesium-sulphate plots and 5 on the calcium cyanamide ones whilst the others varied from 7 to 9. The beetles appeared to be equally abundant on all plots, though no actual counts could be attempted with any degree of accuracy.

F. *Effect of treatments on yield.*

All the plots were lifted on October 15th, and the actual weight of unwashed roots from each of these plots is given in Table I.

It will be seen that the weight of unwashed beet is considerably lower on the control plots (plots A and B) than on some of the treated plots, a high percentage of plants having been destroyed in the seedling stage by the Pigmy Mangold Beetle (Plate XVIII, fig. 4). The control plots with an average yield at the rate of approximately 12 tons to the acre showed that the infestation of the beetle was not exceptionally severe.

The greatest increase in yield is found on plots sown with seed treated with a solution of phenol and magnesium sulphate (plot C). The average weight of beet on these two plots was 7 cwt. 78 lb., compared with 5 cwt. 108 lb. on the controls, showing an increase in yield due to the treatment on an average of 1 cwt. 82 lb. per plot or 3½ tons to the acre (Plate XVIII, fig. 3).

Slight increases in yields were also obtained on plots F and G treated with calcium cyanamide and "drained creosote salts," and on those (plot D) sown with seed treated with mercuric chloride.

Plots H and I, treated with whizzed naphthalene and calcium cyanide, and plot E, sown with naphthalene treated seed, showed a decrease in yield as compared with the control plots. This loss may be due to the fact that the treatments were not effective in protecting the seedlings or that they adversely affected the germination of the seed.

Table I.

Effect of treatments on yield.

		Per plot				Per acre			
		Total yield		Average yield		Average yield		Increased yield over control	
Treatment	Index of plot	cwt.	lb.	cwt.	lb.	tons	cwt.	tons	cwt.
Untreated (control)	A_1	6	26	—	—	—	—	—	—
	A_2	6	2	—	—	—	—	—	—
	B_1	5	84	—	—	—	—	—	—
	B_2	5	88	—	—	—	—	—	—
		23	88	5	108	11	17·8		
Seed treatment:									
Phenol-magnesium-sulphate	C_1	7	46	—	—	—	—	—	—
	C_2	7	110	—	—	—	—	—	—
		15	44	7	78	15	7·8	3	10
Mercuric chloride	D_1	6	2	—	—	—	—	—	—
	D_2	6	80	—	—	—	—	—	—
		12	82	6	41	12	14·6	—	16·8
Naphthalene	E_1	5	0	—	—	—	—	—	—
	E_2	5	8	—	—	—	—	—	—
		10	8	5	4	10	1·4	1	16·4*
Soil treatment:									
Calcium cyanamide	F_1	7	32	—	—	—	—	—	—
	F_2	6	60	—	—	—	—	—	—
		13	92	6	102	13	16·4	1	18·6
"Drained creosote salts"	G_1	5	92	—	—	—	—	—	—
	G_2	6	42	—	—	—	—	—	—
		12	22	6	11	12	3·9	—	6·1
Naphthalene	H_1	5	74	—	—	—	—	—	—
	H_2	5	52	—	—	—	—	—	—
		11	14	5	63	11	2·5	—	15·3*
Calcium cyanide	I_1	5	64	—	—	—	—	—	—
	I_2	6	20	—	—	—	—	—	—
		11	84	5	98	11	15	—	2·8*

* Decrease.

It is doubtful how far the increases obtained with some of the treatments tested can be regarded as significant except perhaps those with phenol and magnesium sulphate and with calcium cyanamide; these mixtures have apparently benefited the crop. It is obvious, however, that the value of any treatment cannot be decided from the results of one or two years, but only from those obtained over a succession of seasons and in varied soil conditions.

(2) *Field experiments*, 1931.

In order to obtain more conclusive evidence of the value of the phenol-magnesium-sulphate treatment, a further experiment was carried out in 1931 at Leighton, Shropshire, in a 5-acre field where an early sown crop of sugar beet had been a complete failure as the result of an attack by the beetle. The variety of sugar beet used was Kuhn, P. and the seed was drilled in 19 in. rows in the third week of May at the rate of 18 lb. to the acre. Two $\frac{1}{4}$-acre plots were sown with untreated seed and served as controls, while the remainder of the field was sown with seed treated with phenol-magnesium-sulphate solution.

The seedlings on the areas sown with treated seed appeared above ground some two or three days in advance of those on the controls, and this advancement in germination was accompanied by a quicker rate of growth throughout the seedling period. As the season progressed this difference in growth gradually became less apparent, and by the end of July was no longer evident. Though the beetles were present in large numbers throughout the field irrespective of treatment and destroyed a high percentage of plants in the seedling stage on the control plots, no such damage was observed where treated seed had been used.

The sugar beet was lifted during the first week in November and the actual yields obtained on the two control plots and those on two plots of similar size in the area sown with treated seed are shown in Table II. The calculated average yields per acre under both treatments are also given together with the apparent increased yield per acre as the result of the treatment.

Table II.

Effect of treatments on yield.

		Per plot ($\frac{1}{4}$ acre)				Per acre			
		Total yield		Average yield		Average yield		Increased yield over control	
Treatment	Index of plot	tons	cwt.	tons	cwt.	tons	cwt.	tons	cwt.
Untreated	A_1	1	16·92	—	—	—	—	—	—
(control)	A_2	1	19·73	—	—	—	—	—	—
		3	16·65	1	18·33	7	13·32	—	—
Treated seed	B_1	3	6·34	—	—	—	—	—	—
	B_2	3	3·79	—	—	—	—	—	—
		6	10·13	3	5·06	13	0·24	5	6·92

It will be seen from Table II, that the seed treated plots gave an increase in yield over the controls on an average of 1·34 tons per plot or 5·36 tons per acre. The average number of plants lifted on the control plots was 5750 compared with 7612 on those sown with treated seed. The average weight of individual roots on the former was, therefore, 0·75 lb. and on the latter 0·96 lb. The difference in the number of plants lifted is explained by the fact that a high percentage of plants on the control plots were actually destroyed in the seedling stage by the beetle. The difference in size of the roots, on the other hand, may be attributed to a beneficial action of the seed treatment in stimulating a more rapid rate of growth throughout the earlier stages of development of the plant or to some more direct control of the beetle.

XI. PRACTICAL ASPECT OF TREATING SUGAR-BEET SEED WITH PHENOL-MAGNESIUM-SULPHATE SOLUTION.

In practice, the application of this treatment occasions no difficulties. A quantity of water suitable for the amount of seed to be treated is poured into a tub. To every 10 gallons of water, 1 lb. of phenol or carbolic acid crystals and 5 lb. of magnesium sulphate are added. The seed is placed in a number of small thin bags and allowed to stand in the solution for about 20 min. with occasional swaying to and fro. After steeping, the bags containing the seed are hung up over the solution and left to drain. It is not necessary to steep all the seed at once since the same solution can be used several times. A large bulk of seed can, in this way, be treated with a minimum quantity of solution. Difficulty is sometimes experienced in obtaining carbolic acid crystals, and carbolic acid solution or some other form of carbolic acid may be offered, in which case it is necessary to use the equivalent of 1 lb. of carbolic acid crystals in 10 gallons of water.

It is found in practice that treated seed will drill without any difficulty even after a very short period of draining and that, if it becomes imperative, the treated seed can be successfully stored provided it is first of all spread out to dry in order to safeguard against heating. In one instance, seed treated in this way was stored for a fortnight and then germinated well.

XII. CONTROL MEASURES IN RELATION TO OBSERVATIONS ON FACTORS DETERMINING INCIDENCE OF ATTACK AND ON RESULTS OF FIELD TRIALS.

The only practical means at present known of exterminating the Pigmy Mangold Beetle, once it becomes well established in areas devoted to sugar beet or mangolds, is by starving it out. This can only be affected by keeping the ground free from sugar beet and allied crops for a number of years. The exact length of time that is necessary cannot be definitely stated, but, judging from the field observations already described, an interval of two years may often be sufficient, though cases are known of satisfactory crops of sugar beet after a rest of one year only. In adopting this measure of control, it must be borne in mind that the beetles may live unnoticed during the interval between successive crops upon the weed Fat Hen (*Chenopodium album*) and even on the debris of sugar beet or mangold. Further it would appear that susceptible crops should not be sown in close proximity to previously infested areas.

Where frequent cropping of infested land with sugar beet or mangold cannot be avoided, the following measures are recommended:

(*a*) *Encouragement of rapid growth.* The first essential is a good preparation of the land, together with a generous application of a nitrogenous fertiliser in conjunction with farmyard manure or complete minerals, in order to stimulate growth and rapid root development of the young plant. The nitrogenous fertiliser should be harrowed in with the seed or applied to the land some time before the seed is sown. It must be remembered that, so far as Pigmy Mangold Beetle is concerned, this fertiliser is chiefly needed during the early stages of growth, and may be harmful if used too freely, as it will tend to retard the maturing of the plants and formation of high sugar content.

(*b*) *Treatment of seed.* In view of the results obtained in the field experiments described in this paper, the seed should be immersed for 20 min. prior to sowing in a solution of 5 parts magnesium sulphate and 1 part phenol in 100 parts of water. The seed should then be drained and sown with as little delay as possible.

(*c*) *Thick seeding.* High rate of seeding is advantageous to compensate to a certain extent for the seedlings destroyed by the beetles.

(*d*) *Consolidation of the soil.* It is desirable to obtain a firm seed-bed and to roll the land frequently throughout the period liable to attack by the beetle since plants usually suffer less injury from infestations under these conditions.

(*e*) *Late thinning.* When an attack is in progress, singling or thinning out of seedlings should take place at the latest possible date with due regard to climatic and soil conditions.

XIII. Summary.

1. An extensive investigation of the Pigmy Mangold Beetle (*Atomaria linearis* Steph.) on sugar beet and mangold has been made during the past six years, both in the field and in the laboratory.

2. The symptoms of attack are given, together with a brief account of the distribution and life history of the beetle.

3. General field observations of infested areas both in Lincolnshire and Shropshire indicate that epidemics of the Pigmy Mangold Beetle in sugar-beet and mangold crops only occur under certain conditions, of which continuous cropping of the land with susceptible plants is usually considered of primary importance.

4. It is found that environmental factors have a marked influence on the relative susceptibility of sugar-beet and mangold crops to serious injury by the beetle. Evidence is produced to show that many of these factors are under the direct command of the grower and their significance on control measures discussed.

5. Field experiments on control of the pest show that, of the treatments tested, the use of phenol and magnesium sulphate at the rate of 1 and 5 per cent. respectively, as a seed steep is the most effective.

6. There is reason to believe that the common practice on the Continent and in certain districts in this country of sowing crude naphthalene with sugar beet seed as a deterrent against the Pigmy Mangold Beetle results in serious damage to germination.

7. Different varieties of sugar beet vary in the degree to which they are liable to infestation but the differences are generally small and unimportant. Mangolds, as a rule, are more resistant than sugar beet.

8. In addition to mangold and sugar beet, the beetle is recorded for the first time in Great Britain on garden beet and White Goosefoot (*Chenopodium album*). Some evidence is presented to show that a rotation of crops as means of control of the beetle may be ineffective in areas where White Goosefoot prevails.

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EXPLANATION OF PLATES XVII AND XVIII.

PLATE XVII.

Fig. 1. Attacked sugar-beet plant; root constricted and finer roots destroyed (see p. 302).

Fig. 2. Attacked sugar-beet plant; root thread-like in appearance (see p. 302).

Fig. 3. Attacked sugar-beet plant; leaves stunted and deformed (see p. 303).

PLATE XVIII.

Fig. 1. View of sugar-beet crop grown on land in close proximity to an infested area (see p. 309).

Fig. 2. View of sugar-beet crop in same field as Fig. 1 but at some distance from the infested area (see p. 309).

Fig. 3. View of sugar-beet crop on plot sown with seed treated with phenol-magnesium-sulphate solution (see p. 312).

Fig. 4. View of sugar-beet crop on plot sown with untreated seed and adjoining that ilustrated in Fig. 3 (see p. 312).

(*Received July 17th*, 1933.)

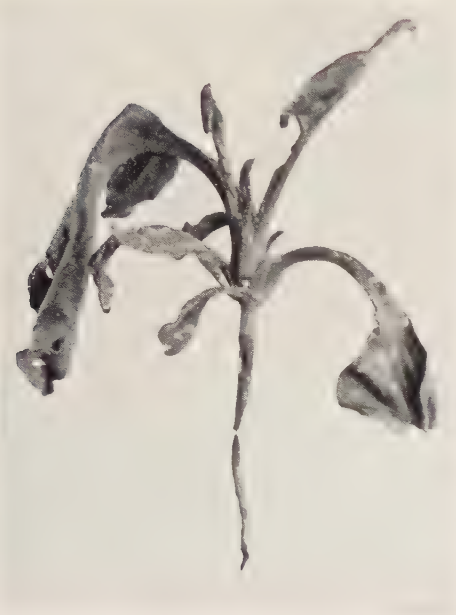

Fig. 1.

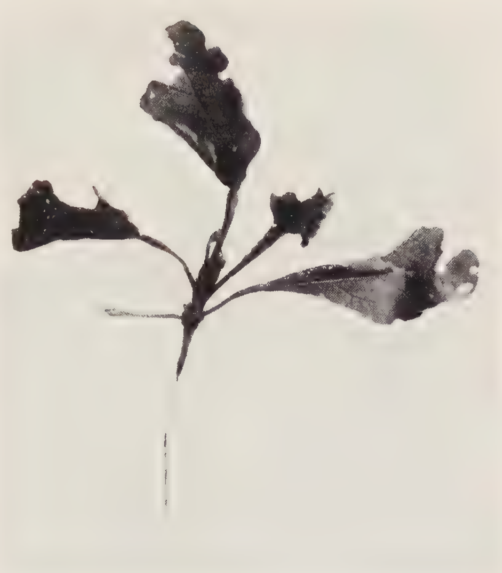

Fig. 2.

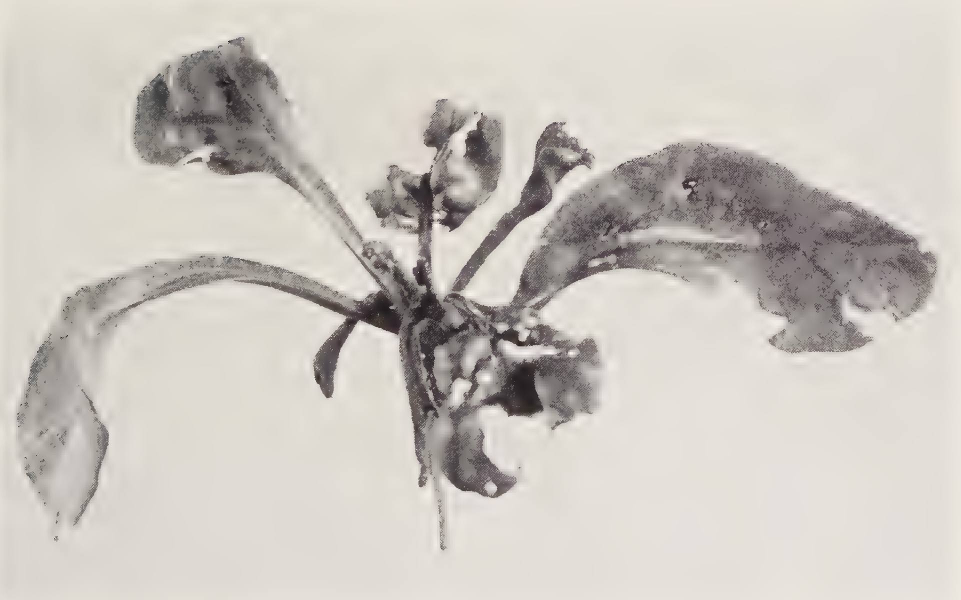

Fig. 3.

EDWARDS AND THOMPSON.—ON THE PIGMY MANGOLD BEETLE (*ATOMARIA LINEARIS* STEPH.) AND METHODS FOR ITS CONTROL (pp. 300–318).

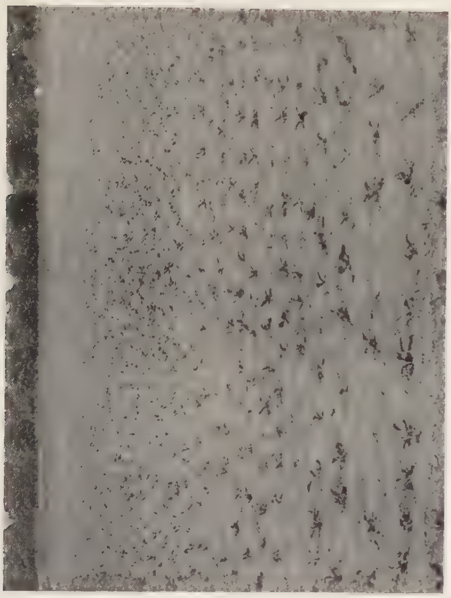

Fig. 2.

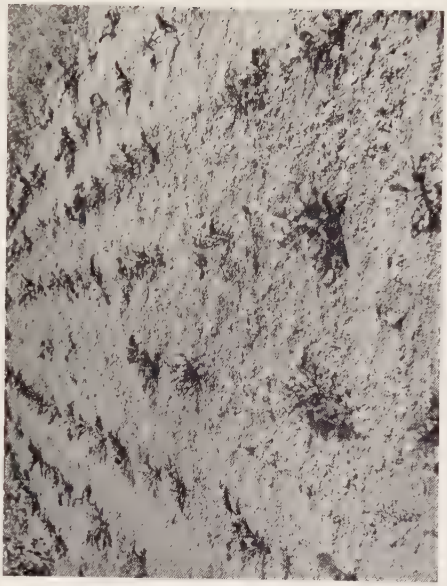

Fig. 4.

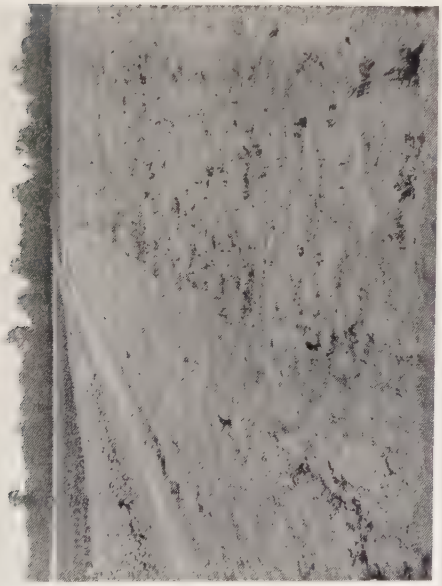

Fig. 1.

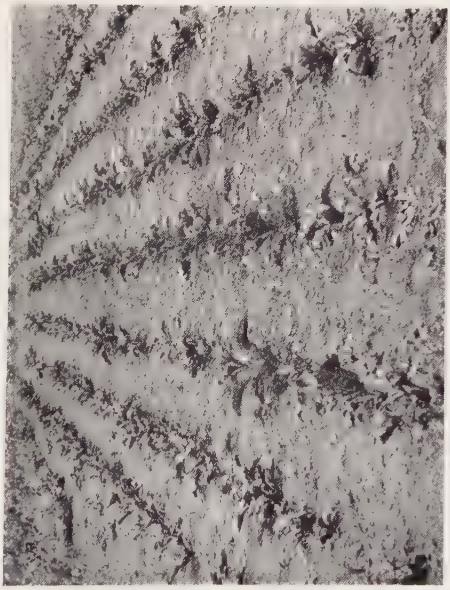

Fig. 3.

EDWARDS AND THOMPSON.—ON THE PIGMY MANGOLD BEETLE (*ATOMARIA LINEARIS* STEPH.) AND METHODS FOR ITS CONTROL (pp. 300–318).

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, December 1st, 1933, at 11.45 a.m. in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Prof. W. B. Brierley.

The programme consisted of a discussion on:

"Physiological Disorders of Glasshouse Crops and Fruit Trees."

The following papers were read:

I. Some Physiological Disorders of Glasshouse Crops. By W. F. Bewley, D.Sc.

II. Some Physiological Disorders of Fruit Trees. By T. Wallace, D.Sc.

III. Injection for the Diagnosis and Cure of Physiological Disorders of Fruit Trees. By W. A. Roach, B.Sc.

I. SOME PHYSIOLOGICAL DISORDERS OF GLASSHOUSE CROPS.

By W. F. Bewley, D.Sc.
(*Experimental and Research Station, Cheshunt, Herts.*)

In considering disorders of plants we find many clearly defined types, but in addition to these there is a wide range of disorders due to physiological causes such as, for instance, adverse soil conditions of texture, moisture or temperature. In consequence of the interrelation of physical factors, these disorders are not easy to investigate.

The subject of my paper embraces a very large field, and I propose to reduce it by dealing with the physiological diseases of the tomato and cucumber only, as these form the chief commercial glasshouse crops.

A rather common disorder of the tomato is Blossom-end Rot. This is first seen as a black necrotic area in the marginal cortical tissue at the blossom end of the tomato. Later a dark brown hard patch shows on the outside of the fruit. The disorder is particularly prevalent on the early crop. The whole crux of the matter lies in the balance in water requirement between foliage and the developing fruit. If the plant has a healthy root system, and the soil is kept sufficiently damp, all will be well, but if for some reason the water supply to the foliage is insufficient, water is withdrawn from the fruit, resulting in the formation of a small necrotic area which later extends until a large portion of the fruit is involved.

In this connection it is essential that the first watering should occur just at the right time.

This disorder appears to be worse when the plants are given an excess of nitrogenous manures. Under these conditions a large amount of top growth is produced in relation to the root system, and it is still more difficult for the foliage to obtain sufficient water.

Another closely related disorder is bronzing of the tissue immediately under the skin.

Until recently this development of necrotic tissue had not been explained, but a series of experiments on soil heating has led us to believe that it is connected with the development of excessive top growth followed by a check due to hot dry soil.

Another common disorder is Blotchy Ripening, sometimes known as Bad Penny, or Waxy Patch. On the fruits appear green patches which may assume a yellow or orange colour during ripening, but never reach the full red colour. At Cheshunt the produce from the various manurial plots was graded, one grade consisting of blotchy fruit. The following results were obtained over a period of years:

Manurial treatment	% blotchy fruit
Complete dressing	1–2
Unmanured	33
Lacking N	10
Lacking K	20

Evidently Blotchy Patch is a nutritional disorder occasioned by deficiency of nitrogen and potash. In commercial work the question of nitrogen-deficiency very seldom arises, and the addition of a potassic fertiliser is the usual remedy. Potash may be present in sufficient quantity in the soil, but for some reason may be unavailable to the plant. Often the addition of a phosphatic fertiliser will assist the plant to take in additional potash.

Blotchy Ripening generally occurs early in the season. To one nurseryman who had previously complained of this trouble the suggestion was made that in some of his houses he should manure the subsoil as well as the top soil. He found no sign of the disorder in any house until August, when Blotchy Ripening occurred in all his houses except those in which the subsoil had been manured with sulphate of potash. In many tomato nurseries manuring is confined to the top 12–15 in., while the subsoil may be in bad condition. The plants flourish until their long roots penetrate into the lower layers of soil, when the later trusses of fruit are affected adversely. Hence treatment of the subsoil is important.

Deficiency of potash and nitrogen affects the foliage as well as the fruit. When potash is deficient the margins of the leaves become yellow: this discoloration gradually spreads inwards and is followed by browning. On the other hand, nitrogen deficiency is indicated by yellowing between the veins, the yellow area slowly spreading outwards.

Grading experiments from the manurial plots also showed that potash-deficiency is one of the determining factors in the disorder known as Green Back. In this condition the fruit develops a green patch around the stalk end. This area remains hard and changes to a greenish yellow colour during the process of ripening.

One other important factor comes into play in regard to this disorder. If a tomato fruit is ripened at a high temperature, say 95° F., the production of the red pigment lycopin is inhibited and the fruit attains a yellowish orange colour. In a similar way, the sun, shining on the top of the fruit, causes a rise in temperature and inhibits the formation of red pigment. This year many more enquiries concerning Green Back have been received than during previous years, and the years 1933, 1929, and 1921, when the disorder has been most prevalent, have also been the sunniest.

The ideal method of shading is to leave sufficient foliage to protect the fruit, but in practice this may not be possible, and the glass has to be shaded artificially. A very good paste for this purpose can be made by mixing 1 lb. of flour with about 2 gallons of water.

Other conditions necessary for the avoidance of Green Back are good root development, moist soil, and an adequate potash supply.

If a good crop of fruit is to be obtained the pollination of the flowers and the setting of the fruit is of primary importance. The disorders associated with faulty development of the flowers are known as Blossom Drop and Dry Set.

The first indication of the former trouble is the yellowing of the tissue at the "joint" just below the flower. This condition shows that the plant is not obtaining sufficient water and usually denotes a dry soil. In actual practice the dropping of the first blossom is a good indication of the need for immediate watering.

Dry Set is the condition arising directly from failure of pollination, generally under conditions of humidity insufficient for germination of the pollen grains. It may be almost entirely prevented by spraying the plants with water as soon as the flowers begin to open.

A most important consideration is the production of high-class fruit which is readily saleable. In this country the public prefers small well-shaped tomatoes, but in the colonies and other parts of the world large fruit used to be popular, and these were often irregular in shape. This is, to some extent, a varietal character, but it is also affected by growing conditions, and good conditions are essential if well-shaped fruit are to be obtained. Occasionally only half the fruit is fully developed. This has been traced to the stigmatic surface receiving insufficient pollen to fertilise all the ovules and points to the need for an abundant pollen supply.

Another complaint is "Hollow Fruit." This disorder is the result of irregular development due to the alternation of periods of rapid and slow growth. Also under these conditions cracking of the skin and splitting of the fruit may occur.

One more disorder that has recently given trouble is Oedema or Dropsy. In this case furry blisters are found on the underside of the leaves and on the stalks of the fruit. Later, leaves and shoots become greatly twisted and distorted. Often after spraying with oil emulsions this condition becomes acute. When oedema occurs the plant is taking up through the roots more water than the leaves can eliminate by transpiration. If the house is well lighted, the atmosphere is kept reasonably dry and watering is moderate there will be no oedema, but in a dull, damp house, where transpiration is bound to be low, oedema may be very troublesome, especially with plants having a large root system in proportion to top growth.

There remains to be mentioned the troubles which may develop during marketing, such as mottling, and softness of the fruit. The former occurs when the temperature is too high so that proper formation of red pigment is inhibited, and the latter is due to bad ventilation in the boxes.

Time will allow reference to only one disorder of the cucumber, namely that which is usually known as "Damping off" of the fruits.

In commercial work the cucumber plant invariably produces more young cucumbers than it can bring to maturity, and a certain proportion of these shrivel at the end and die. This phenomenon is not associated with any organism, but is due to a weakened constitution induced by imperfect root development, root decay or the strain of crop production. When it occurs to any serious extent the plants should be rested by ventilating the houses and allowing the beds to dry. The foliage must be maintained only by "damping down" the house at regular intervals. After 7–10 days of this treatment the plants should be trimmed, the beds top dressed with good compost and watered; the ventilators should be closed and the usual forcing process resumed.

During this resting period new roots are produced and the plant is able to continue fruit bearing for many weeks.

II. SOME PHYSIOLOGICAL DISORDERS OF FRUIT TREES.

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Investigations which have been carried out in the past on physiological disorders of plants have shown that the most common causes of such troubles are (1) deficiencies of nutrient elements, (2) excesses of nutrient elements, including unsuitable ratios of certain of these, (3) unfavourable reaction of the soil, (4) toxic concentrations of salts in the soil, (5) unfavourable water relations in the soil, (6) unfavourable climatic factors.

In many disorders, as encountered in the field, the causes may be somewhat complicated owing to the interrelationships of various factors, but it is usual to find after prolonged investigation of a case that a single factor may be regarded as the primary cause of the malady. Indeed, the history of investigations relating to physiological disorders demonstrates over and over again that knowledge of the cause of a disorder is generally imperfect until a single primary cause can be assigned.

Nutritional disorders of fruit trees occur in every fruit area in the world, and in some countries are of considerable economic importance. A few examples, such as leaf scorch of deciduous fruits, mottle leaf of citrus, rosette of apples and pecans, etc., lime-induced chlorosis, cork and water core of apples, will suffice at this stage to emphasise the economic aspect of the subject which will become clearly evident during the course of the paper.

Investigations of fruit tree disorders present special difficulties which render progress in solving the problems rather slow.

Thus it often requires several years for certain disorders to develop, whilst in some cases considerable periods of time are required to rectify the troubles even when the cause has been ascertained. Again, it is sometimes impossible to overcome the diseased condition once it is well established, and the remedy of the malady to ensure success can only be applied as a preventive treatment in the early stages. Examples of such difficult cases are to be found in leaf scorch (68) and prune die-back (39), where potassium deficiency appears to be the primary cause.

The present account of the nutritional disorders of fruit trees is not to be regarded as complete, but it will serve to illustrate the more important of those on which substantial progress has been made in investigations.

There are many others, but they are often ill-defined, and it is not always even clear whether the primary cause is to be assigned to defective nutrition or to pathological organisms.

A. *Disorders due to deficiencies of essential elements.*

Considerable attention has been given to the physiological disorders of fruit trees which arise from deficiencies of essential elements.

Many of these investigations have been carried out with trees in sand and water cultures with the primary object of extending knowledge of deficiency effects, but in other cases the studies have been actuated by the occurrence of disorders in the field apparently due to definite deficiencies.

At a previous meeting of the Association an account was given of results obtained at Long Ashton relating to deficiency effects on fruit trees, due to deficiencies of N, P, K, Ca, Mg and S(69).

It will thus be unnecessary in the present instance to repeat these but merely to extend the observations where necessary and to give some account of results which have been obtained elsewhere.

Nitrogen deficiency. Deficiency of nitrogen in fruit trees is of very common occurrence, especially under systems of grass culture, but the deficiency is not associated with any well-defined disorder. Trees suffering from lack of nitrogen usually show all-round lack of vigour and fruitfulness, and it is only generally in very extreme cases of neglect that dying back of the tree occurs.

Previously, attempts have been made to attribute disorders such as rosette of fruit trees to nitrogen deficiency at critical periods(62 *a*), but recent work has shown that this is very improbable(9, 9 *a*).

The writer has only encountered one unusual case of nitrogen deficiency in fruit trees, a case where plums and apples were dying out under arable conditions with much rotting of the roots and dying back of the branches, and where the condition was initially ascribed to a root or collar-rot fungus. The soil conditions show the special features of a poorly weathered surface soil, with low content of organic matter, over an impervious subsoil.

The trees have responded well to a heavy application of sulphate of ammonia.

Potassium deficiency. It has been well established in this country that deficiency of potassium is the primary cause of leaf scorch, previously so common in apples, gooseberries and red currants(18, 68, 69, 70, 75), and it has further been shown that deficiency of the element is also the cause of a chlorosis of plums(71).

During recent years potassium starvation has been proved to be the cause of leaf scorch of fruit plants in the field in various countries as follows: Germany and Austria(13, 66, 67, 76)—red currants, gooseberries, black currants; Denmark and Holland(17, 34, 58)—red currants; South Africa(62)—apples. Vogel(65) in Germany and Guildehaus(19) in U.S.A. have corroborated our observations that the ratio N/K is of importance in leaf scorch.

Lilleland(39) and his co-workers in California have associated a die-back of prunes with a deficiency of potassium. The die-back is accompanied by a chlorotic condition and a scorching of the foliage.

Trees affected with the trouble in the field have been improved by heavy dressings of potassic fertilisers, but not completely cured, and have eventually died.

In pot cultures Hoagland and Chandler(32) have been able to grow healthy prune trees in soils producing the die-back when the soils have been adequately treated with potassic salts previous to planting healthy young trees. It is believed that the failure to obtain complete recovery in the field may be due to certain soil phenomena, notably strong absorptive properties for potassium and the presence of high proportions of magnesium and calcium salts. It is possible, however, that the factors of heavy cropping and hot, dry atmospheric conditions are also of importance in the field problem of recovery.

In France with vines, deficiency of potassium has been shown to be causal in cases of brunissure (52, 77, 78), and a chlorosis and stunted growth accompanied by leaf scorch, yellowing of foliage and failure of fruit to ripen (38).

Phosphorus deficiency. Further experiments to determine the effects of a deficiency of phosphorus on fruit trees in sand and water cultures (10, 32) have substantiated the results previously obtained by the writer, but the typical effects of a deficiency of the element have not yet been obtained on trees under field conditions.

Hoagland and Chandler (32) report that they were able to produce typical symptoms of phosphorus deficiency in pot cultures, using peach trees growing in a sandy loam, but state that the deficiency has not been observed on similar soil under field conditions.

Lilleland (39), working with a soil of abnormally low phosphorus content and on which marked responses are obtained from phosphatic fertilisers on such crops as potatoes, vetches and strawberries, could obtain no observable effects on apple trees from phosphorus applications, whilst, on peach trees, the addition of a phosphatic fertiliser appeared on the whole detrimental, an initial favourable response being shown, followed by yellowing of the foliage and symptoms of low nitrogen conditions within the trees.

Calcium deficiency. In sand-culture experiments previously described (69) the writer failed to show any very deleterious effects on apples, black currants and gooseberries due to calcium-deficiency treatment. Colby (10), however, working with French prune in water cultures, has been able to produce very marked results by omission of the element from a complete nutrient solution. The symptoms observed were typical of those which usually occur when calcium is deficient, viz. severe stunting of growth; almost total failure of roots, which tend to turn black; fairly early defoliation, the leaves tending to dry out and wilt and showing slight mottling, otherwise leaf characters remaining fairly normal.

Reed and Haas (54, 55) and Haas (20) have investigated problems of calcium deficiency of *Citrus* under varying conditions in sand cultures, since the element is regarded as of fundamental importance for *Citrus* species. The main effects which resulted from a deficiency of calcium were as follows: roots were poorly developed and young rootlets became gelatinous; shoots were severely stunted and tended to die back; leaves were shed prematurely, frequently curled and exhibited small brown spots, whilst young leaves were often chlorotic; repeated stoppages in growth caused the formation of multiple buds.

Deficiency of calcium with *Citrus*, as in Colby's experiments with the prune, was more serious than deficiency of potassium.

In connection with these results on fruit trees, those of Nightingale *et al.* (43) on tomatoes and of Garner *et al.* (16) on tobacco are of special interest, since effects similar to those observed on the fruit plants were noted. In the case of tomatoes, the upper parts of the shoot portions of the plants were most affected: young growths died, leaves tended to turn yellow and died off; roots were short, bulbous, brown at the tips and somewhat gelatinous; nitrate absorption could not take place in the absence of calcium. Tobacco plants also showed marked weakness near the tip portions of the shoots; leaves tended to curl and failed to develop normally near the margins, whilst in some cases slight mottling occurred.

The marginal effects on tobacco appear to be similar to those described by us for apples (69).

A simple calcium deficiency of fruit trees has not yet been shown to occur in the field, even in *Citrus* trees, although many disorders are known in which normal metabolism is deranged, resulting in apparent deficiencies of calcium in various organs of the plants and more especially in the leaves. Outstanding examples are mottle leaf of *Citrus* (20, 36) and lime-induced chlorosis associated with deficiency of iron (20, 72).

Magnesium deficiency. Further laboratory and pot experiments on this deficiency (10) have served to corroborate the results already reported, and so far no proved case of magnesium starvation of fruit trees in the field has been observed.

A disorder of avocados in which the ratio Ca/Mg is abnormally high has been noted by Haas (21).

Sulphur deficiency. No cases of disorders of fruit trees associated with deficiency of this element have been reported.

Iron deficiency. Lime-induced chlorosis of fruit trees, associated with a deficiency of iron within the plants, is of widespread occurrence in almost every fruit-growing area where considerable supplies of calcium carbonate are found in the soils or rocks from which the soils are derived. Most classes of fruit plants are susceptible to the trouble on calcareous soils, and for this reason much attention has been given both to the practical and scientific aspects of the disorder. On the purely practical side various methods of supplying iron to the plants have been advocated, and the following references will serve to illustrate the main features of the more important of these: spraying with sulphate of iron (7, 33, 49, 64, 73); injection of iron salts into the wood (3, 7); driving of iron nails into the wood (7); dressing pruning wounds with sulphate of iron (64); applying sulphate of iron to the soil in trenches and holes (7, 31); use of permanent cover crops (73).

None of the methods is wholly satisfactory, but certain of them are of commercial value.

Several factors have been shown to be concerned in the production of lime-induced chlorosis in vines in France and Switzerland. Vidal (64) notes the effect of cold in rendering certain varieties susceptible; Ravaz (49) quotes cases in which heavy cropping was considered to have promoted the development of the trouble, and Faes (11) describes the manner in which susceptibility and resistance are affected by root-stocks.

The content of iron within the tissues of chlorotic trees and the conditions affecting the movement of the element in these have been investigated by various workers, notably by Oserkowsky (44, 46), Milad (40) and Wallace (72). The results have indicated that there is no lack of iron within the chlorotic tissues, but that "active" or soluble iron is possibly low. Oserkowsky (46) suggests that a relationship exists in leaves between "active" iron, *i.e.* iron soluble in 1N HCl—and chlorophyll content.

In cases of lime-induced chlorosis, where iron is deficient, ash content is abnormally high in the chlorotic leaves, whilst the ash shows a low proportion of calcium and high potassium (72).

Oserkowsky (45) also states that in chlorotic pear leaves there is a deficiency of yellow xanthophyll pigment in addition to a shortage of chlorophyll.

Manganese deficiency. Manganese has been shown to be essential for the growth of several plants such as spinach, oats, sugar-cane, corn, lettuce, onions, mangels, etc., and in all cases of deficiency chlorosis is developed. Haas (22) has examined the effect of a deficiency of manganese on *Citrus* in water cultures and has shown the element to be essential for healthy growth. Plants grown under conditions of manganese deficiency were stunted, shoots died back and showed gum pockets, leaves were chlorotic and curled, exhibited resinous spots, especially along the mid-rib, and abscissed prematurely. The roots remained in an apparently healthy condition. Deficient plants responded quickly to additions of manganese.

Manganese deficiency of fruit trees has not been reported to occur in the field.

Boron deficiency. The effects of boron deficiency on *Citrus* have been investigated by Haas (23) and Haas and Klotz (27, 28) who have shown that the element is essential for the healthy growth of *Citrus* species. Deficiency results in the following characters: leaves curl downward along the mid-rib, leaf colour being brownish or yellowish green with yellowing along the mid-rib; mid-rib or veins are often corky, conspicuous and split; premature abscission of leaves occurs in a basipetal direction; new twigs die back and there is a tendency to multiple bud formation; the bark of the internodes of the basal parts of the branches may split, from which splits gum oozes out, the woody tissue eventually being exposed; flower production is seriously reduced; roots become dark brown, bulbous, fail to elongate and decay. Boron deficiency in the field has not been observed.

Copper deficiency. A chlorosis of fruit trees occurs in South Africa which has been shown by Anderssen (2) to be due to a deficiency of copper. The disorder occurs on light sandy soils of acid reaction—pH 5·5–6·5. Plums, peaches and apricots show chlorosis, die-back and rosetting; apples exhibit much rosetting and die-back; pears develop dead shoot-tips and severe die-back. The deficiency is reflected in the copper content of all parts of the trees. Treatment with K, Mg, Mn, S, Fe produced no effects on the diseased trees, but they responded quickly to applications of copper sulphate applied to the soil at rates from $\frac{1}{4}$ to 2 lb. per tree. Copper could also be introduced through the leaves.

The disease known as exanthema or die-back, which has long been known on *Citrus* (15), particularly orange and grape fruit, in Florida and California, has recently been shown to be related, if not identical, to a similar die-back of prunes, apples, pears and olives (60). The condition is characterised by gum production, stained terminal branches, die-back of shoots, bark excrescences, formation of multiple buds; branches are often S-shaped and remain immature; the foliage may be abnormally large and dark green, and frenching may develop. The disorder usually occurs on shallow soils where drainage is poor and soil moisture varies between extremes of drought and saturation.

It has been known for some time that the die-back in *Citrus* could be cured by applications of copper sulphate to the soil or beneath the bark or by spraying with Bordeaux mixture, and recently Smith and Thomas (60) have demonstrated that the disease can be controlled on other sorts of fruits by the application to the soil of copper sulphate at rates varying from 2 to 5 lb. per tree. No other fertiliser treatment produced any good effect.

It is not yet established that the disorder is due to a simple deficiency of copper.

Zinc deficiency. The condition of fruit trees known as rosette or little leaf has long been known in the western and southern parts of the U.S.A. (1, 9, 9*a*, 42, 48). All stone fruits, apples, pecans, walnuts, *Citrus*, avocado, and vines are affected. The characteristic symptoms are defoliation of shoots and formation of rosettes of small leaves near the tips of the shoots; the leaves may show mottling or chlorosis, growth is inhibited and the trees may die completely. The disorder appears to be influenced by high temperatures and drought, and these have sometimes been regarded as causal (1). It does not occur in cool, moist climates. Trees suffering from the disorder often recover if grown under cover-crops, especially alfalfa (42).

It has been shown recently by Chandler, Hoagland and Hibbard (9, 9*a*) and by Finch (12) that the condition can be completely remedied by treatment with zinc salts—$ZnSO_4$ or $ZnCl_2$. Zinc, as sulphate, has been used successfully applied to the soil, injected in the trunk or sprayed on the foliage, $ZnSO_4 + Ca(OH)_2$ being used for spraying.

In the experiments of Chandler and his associates, it has been demonstrated that certain soils exhibit high fixing powers for zinc, and hence large dressings of zinc salts are necessary to remedy the rosetted condition. These workers are also of opinion that the case may not be one of a simple zinc deficiency, but that the action of zinc is an indirect one, possibly on the soil flora, which it is suggested produce toxins precipitated by zinc, mercury and silver salts. The point is under investigation.

B. *Disorders due to toxic effects of salts and elements.*

Injuries to fruit trees are common in areas where alkali occurs in the soil and where irrigation is practised (6, 24, 37). In such cases growth becomes stunted, shoots die back, chlorosis or mottling may occur, marginal browning of the leaves is common, and foliage is small and often shed prematurely.

Where salt effects occur the soils are usually characterised by high contents of Ca, K, Mg, Na, SO_4, Cl, CO_3 and $H.CO_3$ (24), and it has been shown in numerous investigations on the subject of salt injury that many of the symptoms observed can be attributed to certain of the above ions (37). Calcium is usually regarded as a favourable ion except when present in the form of carbonate, when chlorosis may be induced. Sodium and potassium are apparently only toxic to *Citrus* when calcium is not available in sufficient quantity (20, 54, 55). Excess of chlorides appears to be responsible for tip burn, usually accompanied by defoliation and killing off of young shoots, whilst sulphate and bicarbonates tend to cause stunting, yellowing, a type of mottling and marked abscission of leaves (21, 24, 29, 37). Irrigation water containing salts and lack of drainage are common causes of salt accumulation in soils, and attention to these factors is usually the best method of dealing with the various troubles.

The constant application of inorganic fertilisers without the addition of bulky manures also causes unfavourable effects on *Citrus*, possibly through salt effects in the soil (63).

In England, after the abnormally dry year of 1921, a peculiar disorder of apple trees, involving scorching and death of many trees, which appeared to be due to a high content of salts in the soil following on drought conditions, was prevalent in the Wisbech fruit area. The condition was examined by Petherbridge, Dillon-Weston and Newman (47), who found total ash and iron contents of affected tissues to be abnormally high.

Excess of chlorine. The rôle of chlorides in alkali soils in the production of tip burn has been mentioned in the previous section. The applications of heavy dressings of fertilisers containing large proportions of chlorides, such as potash salts and kainit, are also frequently the cause of injury to fruit plants, especially small fruits, in this country and on the Continent. The type of injury is usually a marginal scorching of the foliage similar to leaf scorch resulting from potassium deficiency (17, 34, 66, 76).

Excess of manganese. Haas (20) has carried out experiments with lemon, orange, grape fruit and avocado in sand cultures to examine the toxic effects of excess of manganese. The symptoms varied somewhat for the different plants, the main features of the foliage being as follows: chlorosis or a kind of mottling occurred, often followed by a burning of the affected areas; gum deposits usually appeared on the ventral surfaces; with avocado brown stains developed along the mid-rib and veins; the absence of tip burn was characteristic.

Johnson (33) has shown in Hawaii that the presence of high percentages of manganese dioxide in certain soils brings about chlorosis in pineapples, the disorder being a typical iron-deficiency chlorosis which can be remedied by appropriate spraying treatment with ferrous sulphate.

In this case the effect is not regarded as a toxic effect of manganese, but is attributed to the action of manganese compounds in preventing the assimilation of adequate amounts of iron.

Excess of boron. Toxic effects of boron on *Citrus* and walnuts have been shown to occur in Southern California, and in view of the practical importance of the problem extensive investigations have been carried out, mainly on the practical aspects of the disorder, by Kelley and Brown, Scofield and Wilcox, and Haas (25, 35, 59). The two former pairs of investigators dealt especially with the field problem, whilst Haas examined the effects of toxic doses of the element on various fruit plants in water and sand cultures—avocado, lemon, orange, pecan, walnut and peach.

Kelley and Brown, and Scofield and Wilcox attributed the presence of boron in the soils in toxic amounts mainly to the presence of the element in irrigation waters, and showed that concentrations of 1 p.p.m. or greater than 0·5 p.p.m. were liable to lead to toxic concentrations in the soils. Lemon, grape fruit and walnut were quoted as especially sensitive to boron. The element was found to accumulate in the leaves of the various plants. The main effects of the toxicity are as follows: burning appears on the leaves in spots near the tips and between the veins or as a continuous band around the margins, accompanied by chlorosis in certain plants, *e.g. Citrus*; mottling and crinkling may also occur; the element accumulates in the older leaves which, after becoming affected as above, absciss and in this way the foliage becomes thin and sparse; affected leaves show low calcium and high potassium in the ash, like immature foliage.

C. *Disorders associated with unfavourable meteorological factors and deficiency or excess of soil moisture.*

(1) *Disorders of apple and pear fruits—cork, drought spot, water core, bitter pit.* These disorders, mainly in apples, are of great economic importance in the majority of the large apple-producing areas of the world, and in consequence have formed the subjects of extensive investigations (5, 8, 14, 41, 57, 74).

The main conclusion to be drawn from the various investigations is that the disorders are primarily due to drought conditions within the fruits, which may be caused either by excessively high air temperatures or by prolonged drought conditions.

Thus it has been shown that cork and drought spot are most prevalent on thin soils of poor water-holding capacity (41, 57), whilst it has also been proved that dry seasons and high air temperatures may be largely or wholly responsible for the development of cork, water core and bitter pit (8, 14, 74).

Where excessive air temperatures are responsible for the development of the disorders, high soil moisture does not prevent the onset of the troubles (74).

(2) *Disorders of vines.* There are several disorders of vines of which the primary cause is unknown but which are apparently greatly influenced by unfavourable meteorological and soil conditions. Ravaz *et al.* (51) describe a case of chlorosis in which the onset is ascribed to unfavourable weather conditions, drought in winter and spring.

Flavescence and rougeau are stated to be greatly influenced by soil wetness and by drought, especially the former when accompanied by uncongenial weather features such as prolonged wet periods, etc. (50, 53).

Rougeau and sécheresse have also been reported as severe following a prolonged period of drought (61). The disorders occurred particularly on areas where rougeau was previously most severe in abnormally wet seasons. Cultural treatment is recommended.

(3) *Little leaf of* Citrus *in Palestine.* This disorder has been described by Reichert and Perlberger (56), and is quite distinct from little leaf as known in U.S.A. The condition was noted on young *Citrus* trees after an abnormally hot and dry year and is regarded as a form of drought injury. The tree remains dwarfed, leaves are small, brittle and scorched, blossoming is heavy and the fruit misshapen. Analyses of the leaves indicate potassium deficiency but the appearance of the tree is different from an ordinary potassium-deficiency scorch.

D. *Miscellaneous disorders.*

Mottle leaf of Citrus. Mottle leaf is regarded as the most important disorder of *Citrus* (20). Its main characters have been described by Briggs, Jensen and McLane (4). The leaves in the early stages of the disease exhibit irregular chlorotic spots between the veins, the tip half of the leaf being most affected. In later stages, the only chlorophyll remaining is confined to the mid-rib and main veins. The condition is distinguished from chlorosis by the fact that the areas surrounding the yellow spots retain their normal green colour until the condition becomes severe. The foliage becomes thin and weak, leaves are small and the ends of the branches have a bushy appearance. Yield, size and quality of the fruit are seriously affected.

Kelley and Cummins (36) and Haas (20) have examined the composition of various organs of trees affected with mottle leaf. The main points which emerged from the studies were that mottled leaves show apparent deficiency in calcium, and high proportions of potassium and phosphorus and frequently of nitrogen; the deficiency of calcium appeared to be a deficiency of soluble calcium, the proportion of insoluble calcium being fairly normal; in the roots, calcium is fairly normal in amount, but potassium and phosphorus are low. The suggestion is made that, in mottle leaf, amounts of potassium and phosphorus are drawn into the leaves from the normal supplies of the roots. In the main, the special chemical features in mottle leaf are similar to those in iron-deficiency chlorosis.

Experiments by Haas (20) have shown that mottle leaf cannot be considered a simple calcium-deficiency disorder. This worker (*loc. cit.*) has been able to induce mottle leaf in sand cultures of *Citrus* by the addition of toxic materials to the sand—nickel, arsenic, zinc, acid aluminium salts, calcium hydroxide or sulphate—and allowing no drainage water to escape, thus imitating irrigation practice in the field.

The disorder was also artificially produced in sand cultures and in the field by the same worker by the addition of a lithium salt. The leaves showed the characteristic low calcium, high potassium condition of the true mottle leaf (26).

On the fertiliser plots on the Rubidoux site, Riverside Experiment Station, California, mottle leaf developed strongly on all plots receiving nitrogen and especially on the plot which received nitrate of soda only, where after thirteen years of treatment the trees were severely killed back by mottling (63).

In spite of much work the cause of the disorder as it occurs in the field remains unknown.

Decline disease of the date palm. This disease of the date palm is as yet little understood. Symptoms of the disorder are retardation or cessation of growth, destruction of roots and reduction in quality and quantity of fruit. A *Diplodia* is often associated with the condition.

Analysis of diseased plants show low proportions of potassium and phosphorus (30), as also do the soils where the disease is prevalent. One case is recorded of improvement following the application of a heavy dressing of copper sulphate to the soil, 34 lb. in two years.

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III. INJECTION FOR THE DIAGNOSIS AND CURE OF PHYSIOLOGICAL DISORDERS OF FRUIT TREES.

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Introduction.

In the preceding paper Dr Wallace has referred to eleven elements which have been proved to be either essential or of importance to fruit trees. To this list, viz. nitrogen, potassium, phosphorus, calcium, magnesium, sulphur, iron, manganese, boron, copper, zinc, must be added aluminium and silicon, if plants in general are considered. Each of the above thirteen elements has been shown to be essential for the healthy growth of at least one kind of plant; and most of them appear to be essential to all plants. As time goes on this list of essential elements is growing.

Methods for detecting deficiency diseases.

(1) *Water and sand culture methods.*

The essential nature of most of the above elements has been proved by water and sand-culture methods; how productive of results these methods have been was well brought out in Dr Wallace's paper. Simple as these methods are, they are somewhat laborious; they are hardly applicable to large fruit trees; and interpretation of results obtained from their use is complicated by the fact that an increase in the amount of one constituent of the culture solution results not only in its greater absorption by the plant but also in upsetting the balance of the other elements entering the plant.

(2) *Application to soil.*

The apparently simpler alternative, of applying the experimental substances direct to the soil leads to even greater complications in interpretation. As is well known, when a simple substance, such as a potash salt, is applied to the soil, it must, before reaching the absorbing root hairs, first enter the soil solution and get into dynamic equilibrium in the soil solution-soil particle system. A variable fraction of the potash becomes locked up in comparatively unavailable form in the solid soil particles, and in its stead is liberated into solution a chemically equivalent amount of a number of other bases such as soda, lithia, lime, magnesia, etc. So the effect on the solution which bathes the absorbing root hairs of adding a potash salt to the soil is to increase the content of potash by an amount less than that added to the soil and to upset the balance of a number of other bases. The amount of potash which becomes "locked up" varies from soil to soil, and not only the amount but even the nature of the bases liberated may also vary.

An example of similar complications in interpretation connected with the acidic part of salts is furnished by the three types of action so far proved to be exerted by sulphur; in parts of America it is beneficial because of an actual sulphur deficiency, and in others it is beneficial as a result of the liberation, by the sulphuric acid formed from it, of phosphoric acid from insoluble phosphates; in Australia the addition of sulphur has led to the disappearance of manganese-deficiency symptoms, presumably owing to the liberation into solution of manganese from some compound in which it was not available to the plant.

All three methods, water culture, sand culture, and direct application to the soil, break down when it is desired to test the effect of a substance on a plant which does not normally absorb it. Trees of the same variety, but on two different root-stocks, growing in parallel contiguous rows with their roots intermingled in the same soil, were analysed spectrographically; the one root-stock (No. IX) was found to have absorbed molybdenum in easily detectable amount, whereas the other (No. XII) had not absorbed molybdenum in detectable amount.

Besides this qualitative difference in mineral analysis between trees on IX and XII, there were quantitative ones in regard to most of the twenty-odd elements which the spectrograph revealed, viz. potassium, calcium, magnesium, phosphorus, sodium, iron, aluminium, manganese, barium, strontium, lithium, copper, lead, titanium, vanadium, silicon, nickel, chromium, tin, molybdenum, rubidium, and silver. Facts may be found in the literature of the subject suggesting that none of these elements can be safely ignored as being of no physiological importance.

To compare with the differences in content of these various elements are about an equal number of horticultural characters, including vigour, precocity in fruiting, colour and size of fruit, disease resistance, etc. Only the first two characters need be mentioned further. The Lane's Prince Albert trees on No. XII produce enormous trees of upstanding habit which usually do not fruit for about fifteen years; whereas those on IX produce tiny trees of drooping habit which begin to fruit in about two years and fruit heavily in five years. The trees on XII are quite suitable for the Devonshire orchards where trees are not expected to bear quickly, and those on IX are nearly ideal for small bush or cordon plantations in which the heavy labour operations involved in producing highest quality fruit are carried out without the use of ladders. Should their position be reversed, however, each would be looked upon as suffering from a physiological disorder, those on XII in a plantation planned for small trees from a physiological disorder causing excessive vegetative growth and delayed fruiting, and the small trees on IX in grass orchards, where sheep would damage them, from a physiological disorder causing stunted growth.

This special problem, therefore, is sufficiently typical of physiological disorders in general to illustrate some of the difficulties likely to be encountered in detecting the more elusive disorders. We have on the one hand a score or so of elements occurring in varying amount and all of possible importance to the plant, and on the other a similar number of physiological characters, approaching in varying degree the ideal aimed at. When so many elements are concerned, they are not likely to vary quite independently, and so more than one analytical peculiarity is likely to be associated with a particular horticultural character. Thus, even when a suggestive association between a single analytical and a single physiological peculiarity is obtained, it cannot be assumed to be more than accidental until artificially produced changes in chemical composition are followed by predicted changes in physiological character. We have seen that by none of the three methods already mentioned can the amount of an element entering a plant be artificially increased without at the same time modifying the composition of the plant in respect to a number of other elements.

(3) *Tree injection.*

Direct injection of a suitable substance into trees should have obvious advantages. The substance injected would merely supplement the normal supply from the soil and should not upset the dynamic equilibrium existing between the elements in the soil solution. The method should be of the greatest help in studying elements such as molybdenum, which are not taken up by particular trees under normal conditions.

The idea is by no means new, and a bulky literature has grown up around it. Leonardo da Vinci is reported by Stellwaag(4) to have demonstrated in the fifteenth century the presence of arsenic in apples as a result of immersing the cut end of a root in a suitable solution. Before practical advantage can be taken of such a discovery it is necessary to be able to predict not only exactly where the arsenic will travel, but also the parts of the tree which will, with certainty, be free from it. Such information is of equal importance when nutritive elements are injected.

Use of dyes in injection experiments.

In working out the general principles, free use was made of coloured solutions (organic dyes) because of their convenience. Some of these conclusions have been checked by the use of nutrient and other salts in such concentration that they mark their course by producing slight leaf damage. The objections to both these methods are realised, but they are in part met by experiments with salts in non-toxic concentration in which the final distribution was traced by the positive beneficial effects on the tree. Some of these will be mentioned later when the positive effects of injection are considered (pp. 340–2).

Further information along these lines will accumulate as the work goes on, but there is reason for thinking that experiments with suitable dyes reveal the general principles; details of distribution may well vary from substance to substance.

Distribution resulting from tree injection.

The laws regulating the distribution of injected liquids are best illustrated by simple experiments.

If a coloured solution be introduced into a small shallow hole made in the middle of a large straight fruit tree trunk, little or none of it enters tissues external to the cambium, but it passes into the wood and travels in it in all directions. The rates in the different directions, however, vary considerably, the radial, tangential and longitudinal rates of advance being of the order 1, 10 and 100 respectively. An early stage in an injection is shown in Fig. 1 in which penetrated wood is shaded. The longitudinal movement follows fairly closely the direction of the vascular strands; if, as often happens, the trunk is twisted, the penetrated band of wood is in the form of a spiral. The closeness with which the injected liquid follows the direction of the vascular strands suggests that a study of the vascular anatomy of the tree is of paramount importance, but the following experiments will show that pressure, or rather suction, relations cannot be ignored in considering the distribution resulting from injection.

It is common knowledge that the effects of manuring a tree on one side only often show themselves in only one side of the tree-top, which suggests that individual roots normally supply the branches which are in more direct vascular connection with them. In an untwisted tree the affected branch will be vertically above the supplying root, but in a twisted tree they may be at any angle to each other. This well-known phenomenon can be illustrated by immersing the roots of a lifted tree, some in one coloured liquid and the rest in another coloured liquid. The resulting distribution is shown diagrammatically in Fig. 2.

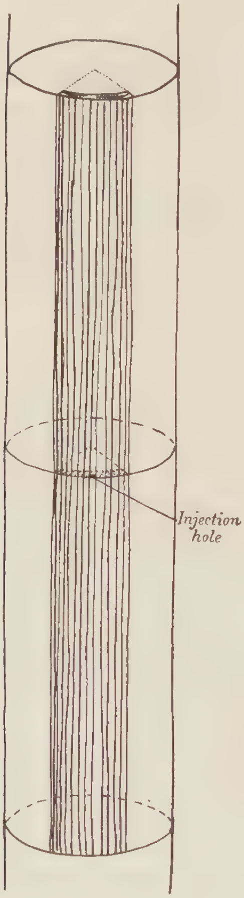

Fig. 1.

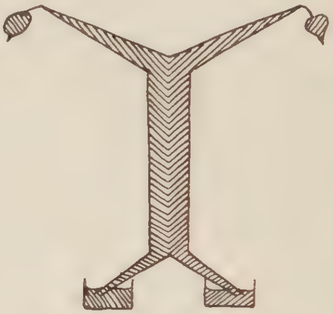

Fig. 2.

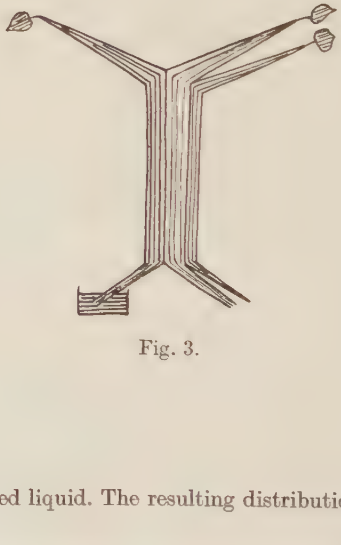

Fig. 3.

If only a single root of another lifted tree be immersed in a coloured solution, the rest of the roots being left exposed, the whole tree, including the exposed roots, becomes permeated with the liquid (see Fig. 3). The early stages of this experiment are interesting. The liquid ascends rapidly along the strands in direct connection with the immersed roots and may even reach the leaves before any moves into other roots and branches; later the result of lateral movement shows itself as a slower permeation of the exposed roots and the branches in direct vascular connection with them. The lines shading these latter roots and branches also represent successive limits of penetration, beginning at their junction with the tissues in connection with the immersed root and ending with the outline most remote from them. The type of distribution resulting from the injection of a standing tree is intermediate between the above two extremes.

Position of hole. Injection through a single hole in the main stem results in the permeation of a band of tissue which in summer rapidly reaches the leaves and the finest rootlets. The type of distribution differs markedly according to the orientation of the hole in regard to the branches. In Fig. 4, the distributions resulting from injection at different points, namely below the junction between two branches and at the point remote from a branch junction, are indicated by shading. It will be seen that injection through a hole at *A* results in the permeation of a band of tissues confined to the one branch; whereas injection at *B* permeates a band of tissues which bifurcate at the crotch; tangential movement in each branch results in the permeation of a corresponding but somewhat narrower band on the opposite side of the trunk. This is made somewhat clearer in Fig. 5, in which the base of the main stem is broadened out into a cone as is done in phyllotaxis diagrams. Fig. 6 shows how, through one well-placed hole, all five branches of a tree may be permeated. (In this branch *A*, having developed from the top, or leader, bud has strands passing down between branches *B* and *C* and between *C* and *D*. The extent of this dominance of the leader branch varies from tree to tree and need rarely be taken into consideration.)

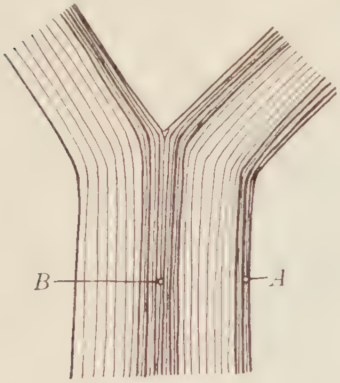

Fig. 4.

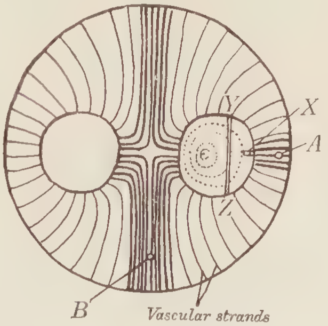

Fig. 5.

(1) *Injection of a whole tree.*

To obtain complete permeation of a whole tree with the minimum number of injection holes, it is obvious that advantage should be taken of the above facts. It is found in practice that the best course to adopt for a tree with a 10 cm. diameter trunk is to drill a hole of ¼ in. diameter through the main trunk with its two ends each under the junction between two branches and so that the hole passes as nearly as possible through the centre of the trunk. Such a tree absorbs from 10 to 20 litres a day in summer. Since ¼ or even 1 per cent. solutions of the common nutritive salts can be injected with safety, and absorption proceeds for from 3 to 7 days before the hole becomes plugged, enough salt may be administered through a single hole to supplement its nutrition quite appreciably. The effects on growth so far obtained will be mentioned later. Elsewhere experiments will be described which suggest that such an injection results in a distribution of the substance throughout the tree, at least as uniform as that resulting from its even application to the soil (see also p. 342).

For smaller trunks, the diameter of the hole may be reduced to $\frac{1}{8}$ in. or even $\frac{1}{16}$ in. with advantage. For larger trees, two holes approximately at right angles are necessary, but the diameter of the holes should not be increased above $\frac{1}{4}$ in. since healing is retarded unduly by such an increase.

The apparatus consists of a glass tube fitting loosely in the hole in the tree trunk. The tube is provided with a collar of sponge rubber sheet about $\frac{1}{4}$ in. thick and fits

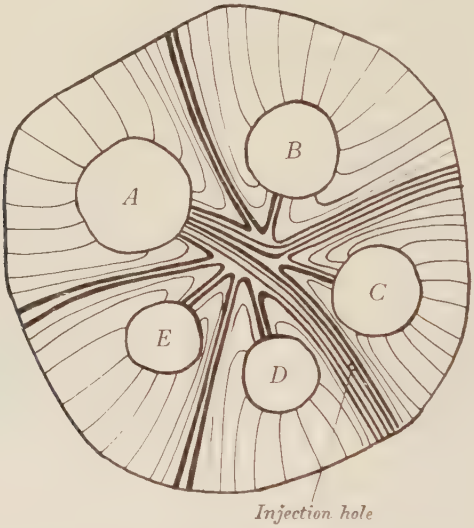

Fig. 6.

into a rubber tube which passes into the reservoir; this should, for convenience, be a foot or so above the hole. A strip about 1 in. wide cut from an old motor inner tube is wrapped round the tree tightly to keep the rubber collar in position, and a similar but unpierced piece of sponge rubber placed over the other end of the hole in the tree trunk. These two pieces of sponge rubber, the collar and the pad, form water-tight joints with the trunk, the surface of which, if necessary, is smoothed with a large knife. It is desirable that the solution should be run in to displace all air from the hole before the rubber pad is finally secured over the hole (see Fig. 7).

(2) *Injection of single branches and their supplying roots.*

If it is desired to inject a single branch without allowing any of the solution to get into another branch, obviously the safest method is to make a shallow hole through the strands most remote from those going to the centre of the crotch, such as *X* in Fig. 5. Such an injection, however, does not result in complete permeation of the branch. If this is wanted, a hole is bored through the branch just above the main crotch and tangential to the main stem (see YZ in Fig. 5). This hole should be just external to the true diameter of the stem in order to reduce the risk of liquid travelling round the smaller inner circles into the centre of the crotch. The fact that the pith lies on the main trunk side of the geometric centre of the branch is convenient in this respect, that the length of ring between the hole and the "inner" strands is thereby increased. The risk of solution passing from one injected branch into another is still further reduced by all the branches being similarly injected, "untreated" ones being injected with water.

(3) *Twig tip injections.*

If an inch or so be removed from the tip of a twig and the cut end placed in a solution, the solution is absorbed and all leaves on the current year's shoot become permeated.

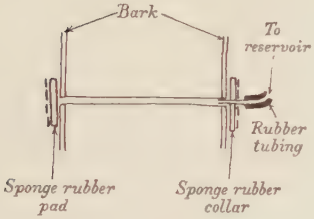

Fig. 7.

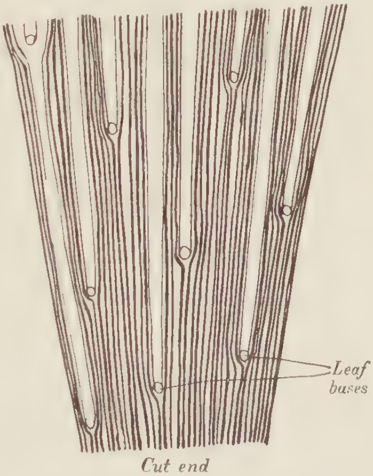

Fig. 8.

More remote leaves are less and less permeated until a point is reached beyond which no solution travels. The reason for this may be deduced from Fig. 8, in which the vascular system of such a twig is indicated in surface view. It is seen that the strands carrying the solution divide and flow on either side of each leaf base, and that the strands supplying the leaves are intercalated between the much divided injected strands. Thus just beyond the third complete whorl the injected strands are divided into no less than eight bands. Since the strands supplying each leaf have on either side of them strands in which the suction tension is lowered, liquid flows from the injected strands into the leaf, which is thus supplied with liquid partly through the cut injected end and partly through the normal channels. It is obvious that as the liquid travels back through the twig, the supply through the normal channels will increase and that through the cut end will decrease owing to the increased resistance offered by the greater length of stem through which it passes until a point is reached beyond which no injected liquid travels.

After a few preliminary experiments with suitable dye solutions on the experimental material, it is possible to predict what length of twig will be completely permeated, and also the extreme length beyond which no solution will travel.

By cutting off greater lengths of branch tips, the liquid may be made to permeate further along the branch. There are horticultural objections to removing more than small branch tips, however, and there is a further limitation to the extension of this method in that no side branch more than about half the size of the main branch at the point of junction is completely permeated. The main use for this method, therefore, is likely to be for injecting current, and perhaps previous, year's wood.

(4) *Injection of branches but not the supplying roots.*

If a branch be injected through a suitable hole, or better, two holes at right angles, say, half-way along its length, all leaves above the injection point will be permeated approximately uniformly, while below the injection point the amount of permeation will decrease in the same way as in the last type of injection, the side branches acting here in the same way as the leaves in the last method described. The greater the number of side branches the lower the injection point may be placed without any risk of other branches receiving any of the liquid. In this way practically whole branches may be injected without any of the injected solution getting into the roots.

Summer and winter injection.

So far, no mention has been made of injection in winter. The same general principles apply as in summer with a few marked differences. Similar trees injected in the same manner in summer and in winter absorb in equal time about fifty times as much liquid in summer as in winter. This greatly reduced rate of uptake in winter is only partially compensated by the somewhat higher concentrations which may be used with safety in winter and the greatly reduced rate of plugging of the opened vessels in winter; whereas in summer absorption usually slows up after three, and ceases after seven, days, in winter absorption proceeds for many weeks.

The most striking difference between the types of distribution resulting from summer and winter injection is shown by main stem injections. We have seen that in summer the liquid travels rapidly into the leaves; in winter, there being no leaves, it advances towards the branch tops more and more slowly as they are approached; even after many weeks of such injection, the liquid injected does not reach the current year's shoots.

Both in summer and winter injected liquid travels most rapidly to the roots in the driest soil; hence it is found that penetration of roots becomes extremely slow when the ground is sodden in winter.

Results of injection.

Only two recent examples of positive results obtained by injection will be quoted, the extensive and largely controversial literature having been summarised by Rumbold(3) and more recently by Muller(2). Andrews(1) as a result of injecting tea bushes with potash compounds rendered them resistant to Helopeltis.

He later attained the same end by heavy applications of the same compound to the soil.

Storey and Leach(5), using a twig tip injection method similar to that described on p. 339, proved that tea bushes were suffering from a sulphur deficiency; later he was able to confirm this conclusion by manurial experiments.

Finally, three types of encouraging results obtained by the writer will be described briefly.

Effect of sodium thiosulphate on apple mildew.

The first experiments concerned the effect of sodium thiosulphate on apple mildew. In the autumn of 1931 root-stocks of type III, which is unusually susceptible to mildew, were removed from the beds before the leaves had fallen and injected with sodium thiosulphate solutions through the cut base of the stem, being afterwards bedded out in the usual way. The six primary infections the following spring were restricted entirely to the ten untreated plants, the eleven treated plants being free. The chances of this disease distribution being accidental is 1/258.

Later in the season the treated plants also became infected. When the experiment was repeated the following year the treated plants were as heavily diseased as the untreated.

In June, 1932, ten pairs of shoots, comparable in regard to vigour, mildew infection, etc., were selected from a large heavily mildewed tree. An inch was cut off each shoot tip and the cut end was fixed under the surface of liquid in a small vessel. In this way ten shoots were injected with $M/100$ sodium thiosulphate and the ten corresponding shoots with water. Six weeks later seven of the thiosulphate-injected shoots were both more vigorous and decidedly less heavily infected with mildew than the corresponding water-injected shoots.

The remaining three thiosulphate-injected shoots were similar to the water-injected ones. The chances of these results being accidental are less than one in a hundred. Later in the season the treated shoots became mildewed.

The slight leaf damage visible in about a day after the thiosulphate injection, the increased vigour visible in the leaves and the length of shoot comparatively free from new mildew infections corresponded with the distribution of light green dye resulting from injecting a 0·1 per cent. solution in a similar manner.

Injections of sodium thiosulphate into various branches of this tree by method 4 in 1933 were not followed by any reduction in mildew infection. The treatment has, then, succeeded twice and failed twice; since the successes were too clear-cut to be accidental, it seems likely that some important condition for success has not yet been recognised. The possibility of a stage in the development of the fungus during which it is sensitive to sodium thiosulphate will be tested by periodic injections. Injection would appear to be the only method whereby such tests can be carried out on different parts of the same tree, and it has much to recommend it for dealing with other types of materials, *e.g.* thiosulphates are rapidly decomposed in soil.

Potassium nitrate injection of a large apple tree.

A fifteen-year-old apple tree was injected with 10 litres of 1 per cent. potassium nitrate solution through a ¼ in. hole passing diametrically through its main stem, but not correctly orientated to give the most uniform distribution. The liquid was absorbed in the course of a hot sunny afternoon. The following day a leaf scorch, indistinguishable from that associated with potash deficiency, had developed. This damage varied in intensity from branch to branch. It was most severe in two branches arising vertically above the injection hole, and least severe in those arising at points more distant from the injection hole. The distribution of this leaf damage corresponded with that obtained later by similar injections of solutions of dyes, in which the leaves were dyed red or metallic green without showing any damage. Only the older fully expanded leaves were damaged, and those expanding after the injection showed no damage. After about a month the leaves took on a darker green colour and were thicker and larger and generally more healthy looking than had ever been seen on the tree before. By the autumn, strong, healthy shoot growth had been made. Measurements showed that, though in previous years shoot expansion growth had been declining, this year it exceeded the previous year's growth by 75, 60 and 50 per cent. in the various branches. The most heavily damaged, *i.e.* those vertically over the hole, showed the greatest increase in growth, and those damaged least showed the smallest increase in growth. Hence the conclusion drawn from the distribution of leaf damage as to the distribution of the salt was borne out by its ultimate effect on growth.

Thus, in spite of the hole having been orientated in a way which later experiments showed did not lead to the most uniform distribution possible, the whole tree benefited markedly in the actual year of the injection was given. The growth increase was, moreover, more uniform throughout the tree than is usually produced by manuring such a tree in the usual way.

Injection of small apple trees with nutrient salts.

In a joint communication with Mr L. A. Thomas, to appear shortly elsewhere, experiments will be described in which various nutrient salts were injected into two-year-old apple trees. In spite of conditions militating against any growth increase resulting from injection, one of 25 per cent. was shown to result from an injection of a mixture of potassium sulphate and ammonium dihydrogen phosphate, the chances being 100 to 1 against the results being accidental.

Some of the facts mentioned in this section and others to be found in the literature suggest that the effects on the plant of injecting a given substance bear a general similarity to those produced by applying the substance to the soil; there is hope, therefore, that with increasing experience the method may become applicable to an increasing number of problems, both theoretical and practical. It is unnecessary to mention more than a few of such possible applications.

When the leaf and other symptoms are sufficiently typical to justify advising the application of potash to a plantation, it is rarely, if ever, possible to say how soon an improvement can be expected; five, or even more, annual dressings are sometimes added before the full benefit is obtained. But if the injection of a solution containing potash into a few trees or branches had given visible proof that the trouble was actually due to potash deficiency, the grower would apply the necessary dressings with the greater confidence.

Plantations sometimes show symptoms typical of more than one deficiency, *e.g.* on a plot at the Research Station, leaves on the same compound fruiting spur showed symptoms typical of nitrogen, magnesium and potash deficiency, in about equal numbers. In a commercial plantation, variation from tree to tree is frequently too great to make a manurial experiment practicable, but it is practically always possible to select comparable branches on the same tree which may be injected each with a different solution. Thus a number of small experiments may be repeated on different trees. Such experiments would be simpler, cheaper, and would probably yield results more quickly than manurial experiments, even were such experiments possible.

It is obviously simpler to apply salts as manures than to inject them, but in special cases it may well be of practical advantage to inject a whole plantation. The slow action of potash on many soils has already been mentioned. Should experiments prove that potash deficiency can be cured in one year by injection, as there is some reason for hoping, the feasibility of injecting a whole plantation might well be considered. The necessary apparatus mentioned on p. 338 presents no difficulties in regard to cost; the manipulation is sufficiently simple to be carried out by the average plantation worker; a tree of average size could be injected at least as quickly as it could be pruned; the necessary water would present no more difficulties than it does for spraying.

Summary.

Water and sand-culture methods are of somewhat limited applicability to fruit trees.

The interpretation of the results of manurial experiments, whether conducted in the field or in pots, are complicated by base exchange and similar phenomena. Direct injection of solutions into trees is, therefore, suggested as an additional method for studying, and possibly even curing, physiological disorders, especially those of the "deficiency" type. Methods for injecting whole trees, separate branches, with or without their corresponding roots, or separate twigs are described.

Some effects of injection on the vigour of the tree and on disease are mentioned.

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REVIEWS

British Economic Grasses. Their identification by the leaf anatomy. By SYDNEY BURR and DOROTHY M. TURNER, with a foreword by Prof. R. G. STAPLEDON, London: Edward Arnold & Co. Demy 4to. Price 10*s*. 6*d*.

The floral characters of the grasses have been fully described and illustrated for a long number of years, so that identification of species on these characters has presented very little difficulty. It must be remembered, however, that there is often considerable difficulty in the recognition of the species as the grasses have a short flowering season and as flowering is more or less prevented in pastures where grazing is heavy. As a result, some other means of identification is required, and this can only be done by a very accurate knowledge of the vegetative characters. With this aim in view and in order to assist the student of grassland the authors have devised a scheme by which the commoner grasses of this country can be identified by their leaves with a large degree of accuracy. On close study of *British Economic Grasses* it appears to have undoubtedly fulfilled its purpose. This is most gratifying, as there has been felt for a considerable time the need of a simple guide to the identification of the pasture and meadow grasses.

A detailed anatomical study of the leaves has been made of over fifty native species. The hand-drawn sections of the leaves are exceedingly well prepared, in that every detail is noted—a task which must have required a large amount of time and painstaking work. The authors have, therefore, been fortunate in having the plates very well reproduced. Alongside the illustrations of the leaf anatomy, there is also a diagrammatic representation of the grass shoot to show the shape, sheath character and the arrangement of the leaves in the bud. Accompanying the plates, brief descriptions are given of the diagnostic vegetative characters and the microscopic characteristics of the leaf anatomy. Preceding the plates and text, two keys have been drawn up, one for the vegetative and the other for the anatomical characters of the leaf. By using these keys in combination and making allowances for a certain amount of variation in grass leaves, there should be very little difficulty in running down any "economic" grass to its species.

When it is remembered that the structural characters of many of the grasses have been studied in Germany and Denmark and also in this country by M'Alpine and Marshall Ward at the beginning of the present century, it is unfortunate that the authors have not made references to some of the more important previous work on the subject. In addition, while one appreciates the difficulties of space in regard to the plates, it would have been a distinct advantage if a standard magnification had been used for the leaf sections. This would have enabled the student to obtain a better comparison between some of the closely allied species.

The book itself is in fact unique and reflects great credit upon the authors. While it will be an invaluable book of reference to teachers and students of agriculture, who are interested in grassland studies and doubtless into whose hands it will find its way, it should appeal to an even wider field of workers in enabling them to get a grasp of a subject very much neglected in the past and yet closely associated with many other aspects of study.

J. S. L. WALDIE.

Recent Advances in the Study of Plant Viruses. By DR KENNETH M. SMITH. Pp. xii + 423. London: J. and A. Churchill 1933. 15*s*.

As natural sciences go the study of plant viruses is one of the youngest: it can, in fact, almost be regarded as a post-war growth. Once, however, its theoretical and practical importance were realised, a large and steadily increasing number of workers was attracted to its study and advance became almost phenomenally rapid. In consequence, our knowledge is not only in a state of considerable flux but is of very uneven quality, and the time had become ripe for the publication of a single work in which the available data and viewpoints were so marshalled and coordinated that one could obtain a bird's-eye view of this recently occupied territory. Numerous workers, during the last decade, have made surveys of larger and smaller areas and there have appeared the more general treatises by Beauverie and by Rivers, but these are now a little out-of-date. This criticism can already be made of the present work, so quickly is knowledge advancing, but it stands to date as by far the most complete and satisfactory presentation of the subject. Further, it has the advantage of being written by an investigator who has made the most weighty contributions to the subject and who is recognised as a leader by all workers. For the student it is a veritable Baedecker, and the research worker in both animal and plant virology will find it invaluable as a handy book of reference.

Chapter I is a general introduction discussing the nature of viruses, their commercial importance, and trends in their present study. It is now generally recognised that symptomatology has been over-emphasised and this was almost inevitable by reason of the nature of virus diseases, but surely there are many other lines of study equally valuable as those suggested by the author. Chapter II is devoted to symptomatology including pathological histology and environmental influences. In Chapters III and IV the physical properties of viruses are discussed, considerable attention being given to the Elford filtration technique. In Chapters V–VII, which deal with insects in relation to viruses, the author is writing on a subject which he has made peculiarly his own and, in some eighty pages, he gives an account of these problems which could scarcely be bettered. It is certainly a remarkable fact that only three of the twenty-three Orders of insects include vectors and that about 90 per cent. of the known vectors are contained in the Homoptera. It is also of very considerable interest that the accumulating evidence apparently tends to negative the idea of any obligatory virus stage in the vector. With the application of Storey's new technique advance should be rapid in our understanding of these fascinating problems. Chapter VII deals with the transmission of viruses by agents other than insect vectors, and with the factors governing the spread of viruses in the field. In Chapter IX is considered the physiology of virus diseases of plants and this is one of the least satisfactory portions of the book. Chapters X and XI, entitled "Some Further Aspects of the Study of Plant Viruses," are very mixed bags but contain a great deal of interesting matter, more especially Chapter XI, which deals with variation and virus complexes. Chapter XII is devoted to potato virus diseases and Chapters XIII and XIV to the virus diseases of other plants, classified according to the host and arranged in the Natural Orders. These last three chapters are not quite up to the high standard of the rest of the book, being written in a somewhat confused style so that it is not always easy to understand exactly what the author has in his mind.

The book opens with a Foreword by Mr Brooks and closes with a short and queerly incomplete glossary of terms used in virus studies, an author index of some 480 names and a good general index.

Each chapter is followed by a full bibliography, citations up to about the middle of 1933 being included. These analysed references are valuable and it is therefore a pity that some of the earlier important papers are omitted, and that the Continental, and more especially the German and less available literature, has not been searched as fully as that deriving from England and America. On the other hand the bulk of virus work is in English, and the Association may take some pride in the fact that over sixty investigations in this field have been published in its Annals.

The book contains 67 illustrations of which the majority are the beautiful photographs which have appeared during the last few years in the Annals. In the Preface the author gives a general indication of the sources of his illustrations but it would have been much more convenient for reference if he had stated the source of each in the appropriate legend.

On the whole the book is extremely well written, but here and there the author has been perhaps a little hasty in his wording. On p. 189, for example, there is the statement that "All plant viruses are transmitted by grafting" a statement which may be true but which requires confirmation and in any case rather begs the question since graft transmission is often regarded as the criterion of a virus disease. The virus nature of some of the diseases included by the author cannot as yet be definitely accepted. On the other hand there are certain diseases such as, for example, Reversion in Black Currant, where the evidence is perhaps stronger than the author suggests.

Misprints have been noticed on pp. 301, 391, 395, and 422, and on p. 201 beet mosaic is regarded as seed-transmissible, whereas on p. 383 it is stated that it does not appear to be seed borne.

The book is a valuable and timely contribution to a difficult subject and all workers in virus and related fields will gladly recognise their indebtedness to the author and congratulate him upon a rather remarkable achievement.

WILLIAM B. BRIERLEY.

Wheat in Great Britain. By JOHN PERCIVAL. Pp. i + 122, 63 plates. Published by the Author: Leighton, Shinfield, Reading, Berks. 1934. 10*s*. 6*d*. net.

"John Percival" is a familiar and honoured name wherever wheat is studied and his monumental volume on *The Wheat Plant*, published in 1921, is a landmark in English agricultural literature. This monograph was based on twenty years' study of wheat and will always stand as a record of personal labour and joy in a task achieved. The material basis of this work was the "Reading Collection" which was Percival's own creation. This living museum which is grown annually in the Agricultural Botanic Garden of Reading University is, outside Russia and the United States of America, probably the most representative collection in existence and includes nearly two thousand races of wheat, derived from almost all wheat-growing countries of the world.

In this collection are some fifty-three distinct wheats which include all the different kinds grown in Great Britain, and the major part of the present work (Part II) consists of a detailed description of these with comments and fifty-five photographic plates containing excellent natural-size figures, each plate showing one race with flat and side of the ear, grains and dissected glumes. Whether for present comparative or historical purposes, it is a valuable thing to have a complete record of the kinds of wheat grown in a country at a particular time, and had such a record existed for previous, even recent, periods some controversy might have been saved. There has been, and still is, much confusion among seedsmen, growers and students regarding wheat synonymy, and these precise and accurate descriptions and clear illustrations should go a long way to obviate any excuse for this in the future, so far as our English wheats are concerned. Part II of the book has been noted first because it is the most valuable portion, having indeed the nature of a contemporary historical document.

Part I, some eighty pages, is a very mixed bag. Chapter I is entitled "Ancient British Wheat," and is an interesting and all too brief account of Roman and pre-Roman wheat illustrated by six excellent plates of wheat and barley grains found in ancient camp sites and lake dwellings. Chapter II, four pages in length, is headed just "Bread," and is an historical note on the cereals used at different times in bread making. Chapter III deals shortly with the chief factors that influence the wheat crop in this country—climate, soil, cultivation, manuring, sowing and seed questions, ripening and harvest and finally yield. In Chapter IV are discussed the distribution of the wheat crop in Great Britain and the import and export of wheat, and there are nine interesting tables which synthesise numerous data. Chapter V is devoted to a brief consideration of the morphological and physiological characters used in the classification of wheat and ends with a note on milling and baking quality. Perhaps the most valuable items in this chapter are Plates VII and VIII which give natural-size photographs of the primary and secondary glumes, from the third or fourth completely developed spikelet of a normal ear, of the fifty-three British Wheats. Chapter VI contains a short and rather inadequate account of the genetic principles underlying the improvement of wheats.

Everyone with experience of wheat growing and, especially, of wheat breeding, will probably find in the book viewpoints and statements with which they cannot entirely agree and which they would like to discuss with the author. This is, of course, almost inevitable when a book is written in a rather personal way about what is probably the most important plant in the world and one presenting innumerable problems for agricultural and biological study. The author's personal viewpoints are, however, the outcome of thirty years' intensive research on living wheat, and the statements are based on first-hand observations. Anyone who does cavil must take care to bring very cogent evidence in his support, for the book is written out of the unique experience of an unusually active and versatile mind.

The general part of the book is written in an interesting pictorial way, with shrewd comments throughout, and each particular topic is treated historically, the development from old to present-day conditions being shown as an organic growth. Here and there in the text Dr Percival mentions authors' names with dates and more rarely the titles of works consulted, but it is a very great pity that he has not appended a bibliography of full citations. There is a short and rather incomplete Index. The book is published privately and is to be obtained from the Author, Leighton, Shinfield, Reading, Berks. The cost of the book is 10*s.* 6*d.* and considering that it contains one hundred and twenty-two pages of text and sixty-three full-page plates the book is cheap at the price.

It is a book that will be found of interest by agricultural students and intelligent growers and seedsmen, and indeed by all who have to deal with this primary crop in a productive, distributive or administrative capacity.

In a private letter to the reviewer the author describes his book as "a product of retirement," but men like Percival do not retire, they give place to successors who are proud to follow them, and then they go on pulling until they drop in harness.

WILLIAM B. BRIERLEY.

Plant Breeding in the Soviet Union. Achievements, Organisation and Future Programme of the Institute of Plant Industry. Published by the Imperial Bureaux of Plant Genetics: School of Agriculture, Cambridge, and Agricultural Buildings, Aberystwyth. 1933. Price 3*s.* 6*d.*

The vast strides in genetic research on crop plants which have been made in Russia during the last fifteen years have amounted to a revolution in agricultural plant breeding. The work moreover has been not only of immense practical value, which is reflected in a rapidly changing crop husbandry, but has entirely reorientated many fundamental genetical concepts. Unfortunately much of this work is not easily available to English readers and the Imperial Bureaux at Aberystwyth and Cambridge have performed a valuable task in issuing this special Bulletin which gives some idea of the position in Russia.

The first part is a brief account of achievements since the Revolution and, in fifteen pages, summarises a vast amount of research. The work has been planned and carried out on such an epic scale that the reading of this mere summary almost stuns the imagination. This first part deals with plant resources as initial material for breeding, the improvement of breeding methods, purity of seed and varietal material, new attainments in genetics and breeding, new varieties, technological analysis of plant varieties, technical laboratory for textile plants, biochemistry, physiology and agrometeorology.

The second part is a brief account of the organisation of plant breeding research in the U.S.S.R.

Part III, headed "The Second Five Year Plan (1933–37)," is divided into two portions, the first being a translation of an interesting address by Vavilov to a Russian Congress held in 1932 and dealing with problems of general and special genetics, whilst the second is a detailed programme for work on the individual plants and problems. The latter is rather like a geneticist's dream, but in view of what the Russians have already achieved by wise planning, efficient organisation and immense industry, all catalysed by Vavilov's genius, it is not unlikely that the dream will eventuate as concrete reality. A short bibliography follows.

The matter recorded in this bulletin is a wonderful tribute to Russian genetical science, and to Vavilov its leader (an honorary member of our Association), and the publication will be of deep interest to all botanists.

WILLIAM B. BRIERLEY.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1933

The Association has met on five occasions during the year, including one field meeting. This was held at the University of Reading and the National Institute for Research in Dairying, by the kind invitation of the Council of the University and the Director of the Institute, to whom the Association is indebted for their hospitality.

The attendance at meetings has been on the average 66 per meeting, of which more than half have been members.

The Council have, with regret, to record the deaths of two honorary members, Prof. Cholodkovsky and Prof. Railliet, and of one ordinary member, Mr Theodore Parker.

Fifteen new members were elected during the year, and the Association now numbers 275 ordinary members and 10 honorary members. Of the ordinary members 223 are resident in the British Isles and 52 in the Empire or foreign countries.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology, and the Council takes this opportunity of recording its grateful thanks, on behalf of the Association, to the College Authorities for this valued hospitality.

The following papers and discussions were brought before the Association during the year 1933.

Feb. 24th. (1) Mr G. M. Wickens: "The stem-rot disease of carnations."
(2) Mr H. H. S. Bovingdon: "Infestation of tobacco by *Ephestia elutella*."

April 28th. (1) Dr S. Dickinson: "The nature of saltation in *Fusarium fructigenum*."
(2) Dr C. B. Williams: "Observations on the desert locust in East Africa."
(3) Mr A. M. Massee: "The strawberry Tarsonemid mite."

Oct. 20th. "Insect and allied pests of cultivated mushrooms."
(1) Mr S. G. Jary: "The incidence of attacks and their relation to growing practice."
(2) Mr M. D. Austin: "Laboratory investigations."

Dec. 1st. "Physiological disorders of glasshouse crops and fruit trees."
(1) Dr W. F. Bewley: "Glasshouse crops."
(2) Dr T. Wallace: "Some physiological disorders of fruit trees."
(3) Mr W. A. Roach: "Tree injection as a research method."

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING DECEMBER 31, 1933

DURING the year ending December 31st, 1933, subscriptions and entrance fees (including arrears paid up) amounting to £306. 8*s*. 6*d*. were received from members. This represents an increase of £14 as compared with the previous year, but it is still below the amount received in 1931 by £33. Income from the sales of the current volume, reprints and contributions to the cost of papers amounts to £724. 17*s*. 0*d*., an increase of £25. The size of the volume of the *Annals of Applied Biology* was increased by nearly 200 pages, and the cost of producing it has risen by £194 as compared with 1932.

Over the whole year there has been an excess of expenditure over income of £32. 11*s*. 9*d*. After all obligations have been met, the assets of the Association amount to £1110. 4*s*. 7*d*., of which £681. 5*s*. 0*d*. is represented by National Savings Certificates. The financial position of the Association is therefore satisfactory; but it continues to be important that members shall pay their subscriptions promptly, and take every opportunity to obtain new members.

J. HENDERSON SMITH,
Hon. Treasurer.

THE ASSOCIATION OF APPLIED BIOLOGISTS.

Dr. *ANNALS OF APPLIED BIOLOGY* INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31ST, 1933. *Cr.*

EXPENDITURE.	£ *s.* *d.*	£ *s.* *d.*	INCOME.	£ *s.* *d.*	£ *s.* *d.*
To Estimated Value of Stock at January 1st, 1933		67 4 0	By Sales—Current Volume		522 1 6
To Cambridge University Press		1081 0 3	By Sales—Back Volumes, Parts and Sets		38 9 10
To Copies Bought in		11 8 9	By Sales of Reprints		159 6 9
			By Contributions towards cost of papers, etc.		43 8 9
			By Cambridge University Press advertisement		6 0
			By Estimated Value of Stock at December 31st, 1933		60 9 0
			By Balance carried down		335 11 2
		£1159 13 0			£1159 13 0

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31ST, 1933. *Cr.*

EXPENDITURE.	£ *s.* *d.*	£ *s.* *d.*	INCOME.	£ *s.* *d.*	£ *s.* *d.*
To *Annals of Applied Biology*, balance brought down		335 11 2	By Members' Subscriptions:		
To Printing and Stationery		12 15 2	Arrears	12 11 0	
To Postages and Cheque Stamps		4 5 9	Entrance Fees	8 18 6	
To Honorariums		18 6	Current	284 19 0	
					306 8 6
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		8 0 9	By Interest on National Savings Certificates and Bank Deposit		26 15 1
To Audit Fee Reserve		4 4 0	By Balance, being Excess of Expenditure over Income for the year		32 11 9
		£365 15 4			£365 15 4

BALANCE SHEET, DECEMBER 31ST, 1933.

LIABILITIES AND SURPLUS.	£ *s.* *d.*	£ *s.* *d.*	ASSETS.	£ *s.* *d.*	£ *s.* *d.*
Sundry Creditors:			Cash:		
Cambridge University Press	348 19 8		At Bank on Current Account	343 8 3	
Audit Fee Reserve	4 4 0		At Bank on Deposit Account	350 0 0	
		353 3 8			693 8 3
Subscriptions paid in advance		13 15 0	Debtors for Subscription 2 years or less in arrear and considered good		42 1 0
Excess of Assets over Liabilities:			500 National Savings Certificates		681 5 0
As Balance Sheet of December 31st, 1932	1142 16 4		Stock of *Annals of Applied Biology* at estimated value		60 9 0
Less: Balance of Income and Expenditure Account for 1933	32 11 9				
		1110 4 7			
		£1477 3 3			£1477 3 3

J. HENDERSON SMITH, *Honorary Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

H. J. COX & CO.
Incorporated Accountants. } *Auditors.*

HARPENDEN, *January 29th*, 1934.

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BY

W. B. BRIERLEY

AND

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SOME VIEWPOINTS OF AN APPLIED BIOLOGIST

BY WILLIAM B. BRIERLEY, D.Sc.

(*Professor of Agricultural Botany in the University of Reading.*)

ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS, DELIVERED TO THE ANNUAL GENERAL MEETING ON FEBRUARY 16TH, 1934.

To an audience a Presidential Address is no different from any other address but, to a President, it is a special occasion, since it is unlikely to recur in his lifetime. He wishes, therefore, to make the occasion notable and seeks a topic of striking and more general interest than his personal research, since this specialised work can, at any time, be laid before scientific societies or published in journals.

In the course of an active life every scientific man accumulates experience, he unconsciously orients himself in relation to certain ideals and values, certain viewpoints and mental attitudes, and he forms ideas about numerous problems cognate to his work or relating to wider issues. These things all remain as a nebulous and secret imaginative background in his mind, rarely crystallising out or issuing explicitly in his trains of reasoning, yet colouring all his thought and exercising an unconscious determining influence on his life and work. I have thought some of them a very proper subject for a Presidential Address and, to-day, I shall try to outline to you in more sharply defined form something of the biological scene of ideas which has gradually been shaping in my own mind. Throughout, I shall try to avoid particularisation and detail and keep to the more general elements in my theme.

Many of these questions are seldom raised, since they deal with fundamentals and their discussion tends to the generation of heat and acute controversy but, if they are to be discussed rationally, they must be brought into the open. On some of them I have tentatively and with some diffidence arrived at certain points of view, but it is not easy to formulate these things in the biological language one is familiar with, or in words which shall be at once explicit and yet not too offensive. If I impinge on anyone's susceptibilities I trust they will forgive me and realise that I gladly admit exceptions to almost every statement I make and that I know myself to be, in many cases perhaps, the best example of those things I most seem to criticise.

The subject and trend of one's thoughts are determined by one's innate prejudices and the set of one's mind. I, personally, happen to possess what I like to think of as a philosophical bias which leads me to an interest in meanings and implications rather perhaps than in doings and things. My friends and colleagues have rudely, but perhaps truthfully, referred to it as an unfortunate and wayward kink tending to ineptitude and sterility; but I hope they are wrong. Whatever it is, it has led me to meditate about such problems as the relationships of scientific disciplines, teaching and research ideals, the cultural value of scientific study and the nature and validity of science itself. It is these questions that I wish briefly and, I am afraid, very inadequately, to consider with you to-day.

It is not always easy to distinguish the many influences which have conditioned the trend of one's thoughts but I am most conscious, perhaps, of the debt I owe to Prof. A. N. Whitehead, to whom I cannot sufficiently express my admiration for the beauty of his writings and my gratitude for their stimulus. Many of his ideas and actual words and phrases will be found scattered throughout the pages of this address, but he must in no wise be thought responsible for the manner in which I have used and misused them.

Relation of "Applied Biology" to "Pure Biology."

Many biologists, and perhaps especially many university teachers, still feel that "pure biology" is, somehow, of a higher scientific and intellectual status than "applied biology." Some of you will remember the meeting of British botanists held in London before the 1930 International Conference when one of our leaders exclaimed: "At this conference let us have pure botany, let us have real botany, we don't want all this applied stuff." And the position was put to me quite sincerely and quite crudely by another eminent botanist at the Conference when he said: "We discover facts and principles, you merely apply them to particular problems, and that is all there is to it." Now institutes of learning are largely immune from outside criticism, and they are not given to self-criticism, so that in spite of the fact that this intellectual snobbery is rapidly dying away, it is still a serious influence to be reckoned with.

For most of us the reasons for doing scientific work are that we can legitimately indulge our "'satiable curtiosity" about the universe, exercise our restless brains, live an interesting life and, at the same time, earn a living: in these things there is no difference between pure biologists and applied biologists.

Most pure biologists hold academic posts. In their research they often formulate a theoretical problem and this problem determines the material worked upon, the subject organism or condition being nearly always of academic and not economic importance. On the other hand, the applied biologist is faced with a practical problem already existing and usually associated with a particular economic organism or given condition. Thus the academic man often moves from his problem to his material, whereas the applied man usually moves from his material to his problem or, perhaps more truly, his material is his problem. Once, however, the problem has been formulated in relation to subject material or conditions, the actual investigation is much the same for both types of worker. The applied is just as much "real" biology as the pure and there is neither higher nor lower degree of scientific or intellectual status.

Further, personal investigation of a practical problem will convince anyone that applied biology is not merely the crude application of academic knowledge to particular situations. Every practical problem is a general research problem in a particular setting, but the setting, being an uncontrolled and often an uncontrollable natural setting, obscures the issue and renders the investigation of infinite complexity. The pure scientist can take the problem out of its natural setting and control and standardise his conditions, eliminating by a highly sophisticated technique most of the difficult factors which must be faced in the concrete problem as it exists in the field. Pure research in the laboratory, museum and herbarium is intrinsically easier than applied research in the field, chiefly because of this increased power of isolating and controlling both material and environmental factors, which allows of the analysis of a general problem into a series of specific problems in artificial settings. This difference sometimes leads the pure scientist, with a certain amount of justification, to be suspicious of the applied worker, for he finds his ideas and results, true of particular controlled settings, interpreted, twisted and modified, and stretched by applications of uncomfortable generality in a way which is very disturbing to him.

It is usually held that pure science is basic to applied science and that pure science, the search for knowledge for the sake of knowledge, is limitless one discovery ever leading to another, whereas applied science leads only to successful application as an end in itself and not to further knowledge. The truth, I think, steers a mid-way course. Science, from its birth, has been a blend or, perhaps more accurately, a mosaic of pure and applied, with the applied acting as a catalyst and suggesting problems for the pure. Pure science has frequently been the key to new avenues of practice, but often it is an explanation and rationalisation after the event. In the main, practice precedes thought, and "the notion of complete understanding controlling action is an ideal in the clouds, grotesquely at variance with practical life." The distinction "pure" and "applied" biology is of course largely an artificial one. Any particular biological study is not either black or white, but mostly shades of grey or mottled, and a botanist or zoologist may be a pure worker at one time and an applied worker at another. Yet, for practical working purposes, there is a broad distinction which we easily recognise although we cannot easily define it, since it derives from viewpoints and ideals, mental bias and differential emphasis, all in their relation to type of problem, choice of subject material, working conditions and objectives. Scientific phase is a result of time and place and "intellectual climate," and sometimes pure biology has been in the ascendant and sometimes applied biology. During the great formative period of organised biology in the second half of the nineteenth century, pure biology largely dominated the scene, but the growth during this century of a vast organisation for research in applied biology has largely restored the balance. For advance in many and wide fields applied biologists find it necessary to lay their own foundations as they go along and, looking back over the period of my own scientific life, I am of the opinion that applied biology has been at least as fruitful as pure biology in opening up new territories of knowledge and giving birth to new ideas and to concepts of fundamental importance.

RELATION OF APPLIED BIOLOGY TO RESEARCH.

Let me say a few words on the growth of applied biology in this country. In pre-war days when applied biology was struggling to recognition, there were the academic botanists and zoologists and there was a handful of so-called economic biologists who were often regarded as of a lower caste—applied biology was a Cinderella. The academic posts have remained almost entirely in the hands of rigidly pure botanists and zoologists who still show little or no sympathy with applied biology and who seem determined, consciously or unconsciously, to maintain the traditions, privileges and vested interests of their caste. It is the result of a natural orthodoxy of the scholarly mind which quite honestly believes that things are best left as they are. I do not think that we can honour too highly those early applied workers whose foresight and devotion, in the face of much discouragement, led to the recognition of applied biology and to the founding of our Association and of our *Annals*. From small beginnings in the early nineteen hundreds there have developed the agricultural and horticultural research organisations, and the various biological services of the Ministry of Agriculture, the Department of Scientific and Industrial Research, the Industrial Research Associations and the tragically deceased Empire Marketing Board. The Cinderella of applied biology has been taken out of her pre-war kitchen and is almost recognised as an equal member of the scientific family.

In Great Britain, and other countries where a similar growth of applied biology has occurred largely outside the universities, there are interesting sequelae. The minute specialisation of research, with a notable gain in expertness and a notable loss of mental stature in the expert; the vast increase in the amount of applied biological research, with its catalytic impact on general biology but with its own incoherent trends; and the greatly increased status of applied biology as an aspect of science are, of course, obvious. More obscure, however, is the tendency which seems to me quite definite, for academic research to dwindle, not in total amount but in man-time amount and, still more seriously, in quality and purpose. It has tended to become devitalised and restricted to the less concrete, less important and perhaps simpler problems of pure biology. This is partly due to the increased prestige of applied biology which attracts many of the best brains, and to the facilities in laboratory, glass-house and field, so necessary in advanced specialised research, which the applied institutes possess, in many cases, far in excess of those of most university departments.

It seems to me quite clear that in biology the universities are becoming increasingly regarded as teaching institutions, whereas advanced research is being concentrated in specialised institutes where little or no teaching is carried out. This trend of things is dangerous both for universities and for research institutions. Teaching, divorced from intimate contact with research, becomes uninspired and repetitive with serious consequences for the immediate students, for the coming generation of investigators and for the teachers themselves. Whole-time research tends to canalise itself too deeply and it moves away from practice and concrete problems. In very many cases it leads to waste of money, time and talent, and to much futility, many workers experimenting merely for the sake of experimenting, with no aim in view, or losing sight of the end of the research in pursuit of the means. Observation and experiment are important, but far more important is original and creative thought, a quality which is so singularly lacking in much of our research to-day. The experimental obsession, which so often leads to the promiscuous collection of data and the purposeless accumulation of trivial information, has usurped the throne of science and has almost completely obliterated the basic value of thought and theory which alone can guide its work and give order and significance to its results. The word "research" has assumed an aura of sanctity which hallows much futile irrelevancy.

It is sheer delusion that there is inherent value in the mere collection of facts, and we do not so much merely need more research as research in the right directions, research guided by imaginative vision and inspired intuition, systematised by creative original thought and synthesised by wisdom. The man who possesses that constructive vision and luminous quality of mind which fits him to carry out whole-time research fruitfully for more than a limited period is a very rare man indeed.

I feel sure that it would be advantageous to both sides if all biological research institutions were linked intimately with universities; if the research staffs carried on a certain amount of elementary and advanced teaching, and if the teaching staffs and students carried on their work in an atmosphere of research, especially applied research.

And whilst I am speaking of research institutes, both those at home and those abroad, let me say as a considered opinion that I regard their hypertrophic growth tendencies as very unfortunate, although perhaps inevitable. Keen men always seem to want to expand, and energies that should be devoted to the furtherance of knowledge and increased joy and efficiency in practice are absorbed and dissipated in the running of the research machine; all of course rationalised as for the advancement of science. The value of the research ceases to be regarded as the criterion, and expansion of income, buildings and staff becomes an end in itself, to the greater glory of the institution. Subjects, posts, capital and maintenance take on the nature of permanent establishments, and become vested interests, and so the vicious circle goes on. The ideal research institute seems to me to consist of minimum and largely temporary laboratory buildings, maximum glasshouse and field facilities, mobile subjects and posts, and temporary organisation throughout.

Moreover, the finances of research are not infinitely extensible in any country and if available monies are earmarked for large and well-established institutes crystallised in particular settings and buttressed by tradition, it seems clear that new and potentially valuable developments, which demand a radically different setting, may remain stillborn. Stereotyped organisations and vested interests have no legitimate place in scientific research, but once a scientific institution or organisation has become established it seems almost impossible to do away with it, or to effect any fundamental change in it, although the original reasons for its establishment or particular shape may no longer exist. Organisation in research there must be, otherwise research would come to an end, but we need to give far more thought to what is, at any particular time, the best organisation for research activities and how that organisation can be maintained in its most fluid condition and changed or replaced as need arises.

Moreover, we give little thought to the intimate details underlying the organisation and routine of our scientific laboratories, most of which are conducted in a way that would drive a business firm into immediate liquidation. We do not in the least want business ideals in our laboratories, but the introduction of a little business method in our routine, such as is found in efficient commercial laboratories, would be a vast improvement and would eliminate what, in the aggregate, must be a fabulous waste of time, money and mental value. It is a waste that our country and our science simply cannot afford.

Further, we need to pay far more attention to the method of recruiting our research workers, which at present is a very haphazard affair, depending largely upon the possession of academic distinction, which is by no means synonymous with research ability. Real research minds are rare and, when such men are found, they should be supported adequately and freed from the burden of routine administration which dulls their edge and increasingly absorbs their time and energies.

Also we have paid little attention to the bridging of that gulf between research and practice which is one of the major issues before us to-day. The various services of public bodies meet the need to a certain extent, but in view of the size and the depth of the gulf they are the veriest rope bridges, whereas what is needed is a large-scale engineering construction. Apropos of our own immediate part in this, it seems to me quite clear that our scientific results must be translated into intelligible language and explained to the layman. Science for the scientist seems to me totally objectionable, leading to the not unlikely danger that research men will develop into an esoteric and priestly caste holding a body of secret knowledge. Scientific results show their highest value by becoming the property of the people.

And, finally, I cannot help wondering whether, in our research institutes and perhaps even more in our universities, we do not place an excessive value upon peace and quiet, upon placidity of existence, safety of tenure, long-scale grants, immunity from criticism and a freedom[1] of direction and activity, almost amounting to irresponsibility, which is rare indeed outside these institutions. Too great a discomfort or instability is, of course, inconsistent with any possibility of progress, but genius or high talent and striking advances of thought are often the outcome of conflict. In comfort there is much spiritual decay, and we must not mistake increase in comfort for progress or let our science degenerate into a decent formula wherewith to justify or embellish a comfortable life. The life of science is a spiritual adventure often born of travail and nurtured in faith, and the portals of scientific laboratories are inscribed "*Arduus ad solem*" and not "Safety first."

Relation of applied biology to teaching.

From research institutes for applied biology and research ideals let me pass to a brief consideration of teaching institutes and teaching ideals. I believe that the present conditions and curricula in our university departments of botany and zoology are not the best that can be devised, and it seems clear to me that, if we were starting anew, we should construct very different ones. Our biological departments are no worse than other university departments and are simply characteristic of the way in which our higher educational system has developed, and the traditional organisations and conventional modes of thought which prevail. We give comparatively little attention to the "physiological anatomy" of our higher educational system, and the universities themselves are rarely unities, harmoniously integrated systems but, in most cases, mere agglomerations of departments. Many of our university conditions, teaching methods and ideals, and much of our university organisation and general outlook seem to me very astonishing in this twentieth century.

Now buildings, organisation, and certain conditions we cannot easily change, although I think we should not cease from trying, but ideals, curricula and methods we can change and I think we should: the matter is urgent. The immediate questions we have to face are these—what should be our aim in the teaching of botany and zoology, how can we best achieve this aim, and what contribution has applied biology to make to this issue?

Our present scheme is the outcome of fifty years' conventional thought and traditional method and is therefore permeated by the outlook of the pre-applied phase of biology. This domination of our academic outlook to-day by ideas deriving from an earlier period leads, I think, to a wrong balance of values in biological education but, unfortunately, it is a position which all cogent factors tend to maintain.

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[1] I say this with an uneasy mind. Freedom is a treasured possession and I would admit no compromise whatever regarding freedom of thought and freedom of speech.

Whether a student is going to teach one or another branch of biology, whether he is going to become a research man or specialist, or whether he is going to be a general practitioner in the field or a practical man of affairs it seems to me imperative that, in his general training, he should realise something of the potentialities which his subject holds for human welfare. It is said that this is specialised knowledge which a student can only appreciate at the end of his general fundamental training, but this is, I think, an academic inversion of values. The principles and data of applied biology seem to me more generalised and fundamental, and a great deal more vital, than much of the extremely specialised and rather remote knowledge which students now imbibe during their undergraduate years.

It has also been pointed out to me that it is impossible to fit anything new into the curriculum since students cannot even carry their present load. Professors and students have always protested thus and I agree. In the ontogeny of each student's training, we still try to pack the phylogeny of the subject. Ontogeny may recapitulate phylogeny but, if it does, it is in a terrifically shortened and modified way, and so in our academic teaching we must eliminate, modify and condense to achieve our aim. We must not, however, condense by eliminating entirely the humane aspect of our discipline but rather by exercising a more stringent selection and by modifying and balancing throughout.

The viewpoint sometimes adopted that "it doesn't matter what or how we teach our students" seems to me just frivolous and unworthy of consideration.

The present teaching is almost entirely in the hands of traditionalists safely nourished from their student days on scholastic values, growing up within one or two academic institutions, rarely leaving the pale of academic life and having little knowledge and less experience of the life and work of the outer world. Such teachers will naturally see every reason for maintaining the safe scholastic tradition, for it is their own tradition, a lifetime's faith to be defended against heretics and barbarians. Most men of middle age and senior position become increasingly conservative and innately antagonistic to any innovation disturbing to their habitual modes of thought or demanding deep and far-reaching alterations in the organisation of their familiar institutions and the balanced conditions of their lives. This is not with any deliberate obstructive intent but is, largely, simply a failure to appreciate either the need for change or the tentative efforts of the reformers. Twenty-five years' very mixed experience of biological teaching and research has made clear to me that the scientist is no exception to this rule. Men's vision is dimmed by the sincerity of their faith and, so honest is prejudice, that men have always been able to defend their self-esteem and the conditions supporting it under the impression that they were defending their faith. I cannot do better than to ask botanists to look at the correspondence on the "Reconstruction of Elementary Botanical Teaching," published in the *New Phytologist* for 1917 and 1918 where a plea for a simple, perfectly obvious and quite harmless innovation which was long overdue and which has since been generally accepted was opposed and finally described as the advocacy of "immediate botanical bolshevism." I shudder to think how the ideas I am putting forward to-day may be described.

I entirely agree that students should receive a sound fundamental training, but I hold that certain aspects of the present curriculum have a false and traditionally exaggerated value in that training and that, if they were condensed and subordinated, it would be possible to include other aspects, at present omitted, which seem to me essential. In the growth of any scientific discipline there occur periods when it is necessary to revise ideas regarding both the content of the subject and the best methods of teaching it. Much of traditional biology has only historical significance for us to-day and, in the historical perspective, has interest and value. In our teaching practice, however, it is an accumulation of dead and moribund matter obstructing our stream of thought and it ought to be swept aside. The very fact that during this generation we have built up and accepted a great organised development of applied biology has already committed us to educational change of a far-reaching order, with all the modification and disruption of traditional forms which this involves. It is, surely, quite clear that applied biology is an integral part of the vital thought and culture of our age and, as such, must be incorporated in any constructive system of higher education.

I cannot believe that to-day a biological education can be fundamentally sound and reflect our "Weltanschauung" if it excludes from the students' vision some understanding of the distribution, structure and development, genetical, vital, morbid and death processes, and human values, of economic plants and animals. The study of those plants and animals on which mankind depends for its very existence, or of those organisms and conditions which menace that existence, is not a specialised pursuit for a few experts only, it is an integral part of any biological training. Such study is fundamentally heuristic, giving full scope for observation, experiment and reasoning and appreciation of scientific method, and is far more valuable than much of our present academic curriculum in developing a scientific conscience, vigorous and original thinking, and a balanced and cultured mind.

To a degree we are tyrannised by words. "Pure Botany," "Pure Zoology," "Fundamental Training," "Research," these, and other traditional slogans, are surrounded by an aura of sanctity and carry a mystical prestige. Whatever language we use, all we want is that the student, whatever he may become, shall, whilst *in statu pupillari*, attain some understanding of the principles of animal or plant distribution, structure, functioning and relationships and, in the attaining shall acquire the scientific outlook and develop such talents and ideals as shall fit him for life in the world of *to-day*, and enable him to justify his existence by contributing to his intellectual and social milieu.

Now the academic biologist tends to look at this educational process from a purely intellectualist standpoint, seeing the end as the production of an adequate botanist or zoologist *qua* botanist or zoologist, the judgment of adequacy being according to the scholastic criteria of academic tradition. I have the greatest esteem for scholarship and love of learning, but scholarship for the sake of scholarship only, and thought divorced from life leading to life as pure intellectualism only, seem to me not only high abstractions but inherently unreal and fantastic ideals. No one will think that I am advocating anti-intellectualism but rather an intellectualism rooted in experience and integrating throughout with the ordinary stubborn facts of daily life. Life is wider than logic and logic must be justified in terms of life. Critical detachment and objectivity are essential in the search for truth if self-delusion is to be avoided, but it must be realised that they are means and not ends, that they are single elements only in creative activity. In much modern biology they have been pursued so exclusively as almost to deny the creative unity of life. We are not pure intellects but men and women living in a work-a-day world, and all our contacts are human contacts, and all our knowledge and ideals are human knowledge and human ideals: "pure knowledge" only exists as an abstraction in the minds of men, a sort of intellectual mirage. I quite clearly recognise that in our study we must simplify and abstract since analytic science would be impossible without such abstraction. The danger arises in the fact that when we try to interpret and to synthesise our results we forget their abstract nature and, quite unconsciously, adopt the "fallacy of misplaced concreteness."

Every biologist knows that an organism cannot be isolated from its environment, it can only be given a different environment, and so a biologist cannot conceive *pure* organism, but only organism in relation to environment. In order to understand the life and functioning of an organism one may standardise and control the environment, practising a Baconian technique, but this is all to the end that one shall understand the organism in its natural setting, which is the concrete problem. And so with knowledge: one may intellectualise it into the abstract logic of pure science, standardising and controlling its environment, but the concrete problem is knowledge in its immediate human setting with its nature and values exemplified in the daily life of men and women.

The relation of all this to the question of the teaching of biology in our universities is this, that to guide the student to a purely intellectualist ideal, as we tend to do at present, is to mistake a means for an end, to leave the student facing the mental apparatus instead of facing the concrete problems. The least we can give our students is a glimpse of biological reality in the human environment and this can best be done by incorporating applied biology in our curricula, not as a new or additional subject but as a change of emphasis throughout, leading to a new balance of scholarly values. At present we train minds to conform to a methodology, whereas what I ask is that the methodology shall be recognised for what it is, not an end but a means to an end, as merely one element in a larger synthesis. We are more interested in instruction than in education and, in consequence, our training is *for* botany or zoology, whereas it should be an education *through* botany or zoology for life. We allow the demands of professionalism to override and obliterate the claims of a liberal education, so producing skilled technicians and not educated men and women. And in teaching the peculiar routines and narrow results of our special sciences, neglecting their humane application, we entirely overlook the fact that our biological teaching is only adequate when it brings biology into relation with everyday life.

In closing this portion of my address let me point to the essential contribution that applied biology has to make to teaching method and teaching ideals. I have urged that the human setting of biological knowledge can best be exemplified by a changed emphasis in the study of biology. Our biological teaching is strung along single threads which are the evolutionary principle, but I think it should be patterned as a fabric of which the warp would be the principle of evolution and the woof would be the humane relationships of biology. It is in the broadening and humanising of biology, in its emphasis on a *cultural* attitude rather than a merely intellectual and professional attitude, that applied biology should have its greatest influence in education.

APPLIED BIOLOGY AND CULTURAL VALUES.

One's estimate of the cultural value of an educational system or study depends upon the meaning attributed to the word "culture." To many people of classical outlook, culture implies some degree of personal refinement and mental adornment, whereas to many scientists it suggests, in addition perhaps, some degree of intellectual facility in a particular region of knowledge.

As I have said, pure knowledge or pure science are remote abstractions, all knowledge and all science existing in relation to human life. And, further, no individual is sufficient unto himself, since every individual from birth to death is an integral part of a social and intellectual milieu. I think, therefore, that any *efficient* meaning of the word "culture" must have two aspects, an individual and a social, and I hold the cultured man to be one whose knowledge and experience are so integrated that he can live a reasonably full and free sensory-motor, emotional and intellectual life, and play a contributing part in the social community.

If biology is to have such cultural value it should educate men and women for life and not merely function to produce intellectual ornaments or efficient scientific robots. A zoologist is not a cultured man because he can discourse learnedly of marsupial embryology or control capsid bugs by spraying, nor is a botanist a cultured man because he can discourse learnedly of cycadeoid phylogeny or breed disease-resistant plants.

The cultured biologist is one whose knowledge of the principles and practice of his science is visualised in a perspective of human values, and so co-ordinated by experience that he has achieved wisdom: one who visions life as something richer and greater than the mere knowledge or the mere exploitation of his science, as something to which his science is a means, as something existing according to the fullness and freedom of its living, and to be measured by the degree to which the individual and the world are richer by the living. Science is not weighing on a balance nor peering down a microscope, it is a life to be lived. Science is neither good nor evil, the good or the evil reside entirely in its use, and that use resides in the intention and the mind of man. It is possible to be a brilliant scientist and yet in feeling to be quite uncivilised and, although science must remain morally neutral, we do not want the scientist to be spiritually blind.

And so, in our biological education, we should aim at something between "the intellectual vacancy of the expert" with his gross specialised values and mere scholarship intellectualising its little world in thin specialised values. Wisdom is the fruit of balanced development and integration of personality, and a civilised society can only be created by men and women who are experts in the art and understanding of life and not merely experts in some particular branch of science.

To conclude and draw together these portions of my address, I would urge that our traditional methods of biological education are far too much occupied with intellectual analysis and the acquirement of formularised information; we emphasise abstract formulations and neglect their wider concrete applications, closing our minds to the dangers that attend a self-sufficient quest for knowledge. Advance in expertness and particularisation leads to a better and more progressive performance of the specialised functions of biological science, but the attendant and increasing feebleness of co-ordination results in a growing lack of vision in the generalised direction.

Further "in the modern world of pure science the celibacy of the mediaeval learned class has been replaced by a celibacy of the intellect which is divorced from the concrete contemplation of the complete facts." The complete facts of biology are not in the museum, the library, the laboratory or the herbarium, they are in the fields and woods and waters, in the growers' problems, the industrial and agricultural situations and the concrete problems of nature in its human relationships. And it is not in the laboratory or the museum but in the field of concrete human purposes that biological science must prove its theories and discover its limitations. Professors, especially, dislike intensely the idea that in order to know what the world is really like it is necessary to go out and look at it, and I cannot help thinking that it might vitalise "The frowsty science of the cloistered men" and eliminate some of that complacent indifference of the academic mind to the urgent needs of the modern world, if professors and senior research workers were compelled to spend every seventh year living in the world and earning their living in some practical pursuit, preferably artisan or manual labour.

Botany and zoology, like all other branches of science, must not be regarded as isolated departments of pure knowledge, which is a false and remote abstraction, but as integral parts of human life and social welfare. Mankind does not live for knowledge, but knowledge exists for mankind. And yet in our biological departments, much of our teaching is still carried out as though subject, teachers and students were bloodless abstractions existing *in vacuo*. The inevitable outcome is myopic pedants and rigid specialists who bear little relation to life as it is lived in the world, and pass their allotted span splitting the hairs of academic futilities, mistaking abstractions for concrete realities, and losing all sense of their oneness with the pulsing human life around them. Such biology is not serious, it is a kind of game which certain people play in their scientific nurseries working off their excess mental energy in forms of exercise that have, in many cases, little or no value save as channels of escape. We are not, however, concerned with the playing of games but with the serious business of thought, and with living knowledge, not with moribund or dead knowledge. When biology is taken seriously it not only influences our own immediate experience of living but pervades the whole structure and being of our social existence.

From time to time, and I think that to-day the matter is urgent, we need to take stock of our biological research and teaching, of our principles and of our practice, and to submit the various elements to a searching test of value, rejecting those which fail to survive the test. Obsolete ideals however venerable, and effete practices however widely spread and deeply entrenched, which impede or do not flow with our stream of culture, must be eliminated. In our higher centres of learning, much of what passes for intellectual work is merely frivolous, it has no meaning in our modern world perspective, and only continues to exist by reason of sentimental appeal and traditional value. We need a rational iconoclasm to sweep away resolutely the fetishes, the shibboleths and vested interests which clog the progress of our education and research and we need a luminous and prospective vision to rebuild our educational system in twentieth-century terms—remembering always that acceptance does not necessarily imply acquiescence.

In too many cases mental concentration in pure science merely provides an escape from the realities of everyday life, a retreat from the strenuous public world into a private world where one can more easily and self-satisfyingly control destiny. But the improvement of natural knowledge for use is a duty as instant as that of improving natural knowledge for discovery, and the time has gone by when the scientist could isolate himself and shut out the practical world. Science is a social function with social roots and social consequences and, if our science is to live, it must ever keep essential human values in view and it must justify its existence in the world by its contributions to it. These contributions are spiritual and material and both are equally demanded.

APPLIED BIOLOGY AND SCIENTIFIC VALUES.

In the time remaining to me I want to consider, and it must be very briefly and inadequately, what seems to me perhaps the most important problem before the scientific world to-day, a problem to which all that I have said naturally leads. It is a problem which every thoughtful biologist must face sooner or later, the problem of science itself, its nature and validity, its aim and its future. Science, by its very nature, may be combining various propositions which presuppose inconsistent backgrounds, and no science can be more secure than the unconscious metaphysics which are its foundations. These metaphysical foundations are dogmatically and arbitrarily assumed, are often very inadequate and, by circumscribing observation and experiment and canalising thought within predetermined limits, they may exclude from rational thought some of the final values of existence. Many of the philosophical foundations of science have been changed by the mathematical physicists and clinical psychologists within our lifetime, and I cannot help thinking that many of the explanations of biology are framed in a totally obsolete metaphysics. Biological knowledge has clearly outgrown its earlier metaphysical clothing, but we make no attempt to fashion new garments. Painstaking care and amazing skill and ingenuity are lavished on biological observation and experiment, but the definition and use of the concepts in terms of which our results are expressed are almost completely neglected in our thought. As a consequence, although biological science has made vast progress in discovery, or particularisation, it has made very little progress in interpretation or the formulation of universals embodying those particulars in thought.

We, as applied biologists, must formulate some ideas on these problems; we must have some philosophy, some coherence of thought underlying the work which fills our lives and is the content and intellectual framework of our minds. As applied biologists we approach the natural phenomena of the world from a particular angle, but we are only one line of approach in the general scientific attack and, unless we are merely straws in the wind, we should at least have some idea of the reasons for the attack, the plan and methods pursued and the objectives to be reached. Are the reasons sensible and justifiable, are the plans feasible, are the methods valid, and are the objectives attainable?

Biology means different things to different people, and to most people, perhaps, it means a system of knowledge about living phenomena. To my mind this organised knowledge is a secondary thing, the outcome of the application of the particular method of approach to nature which we call the scientific method. This method is nearly always assumed tacitly by biologists, and so the problems postulated by the method itself, for example, the philosophical and psychological validity of the method as a mental tool, are never even raised in question.

Stated in so many terms I would describe the scientific method as follows: firstly, observation; secondly, perceptual appreciation of relations among the observed objects or conditions; thirdly, inductive reasoning leading to the formulation of a working hypothesis, that is, conceptual relationship; fourthly, experimental testing of the hypothesis; and fifthly, the use of the confirmed hypothesis to make a generalisation which, in turn, becomes a working hypothesis in a wider relation, and by means of which future particular facts may be predicted. Present scientific developments tend to raise the predictive quality (statistical probability rather than strict predictability) of a hypothesis, especially if that hypothesis be consilient with the rest of knowledge, to a rank equalling in methodological value that of experimental confirmation, a point of primary importance in the consideration of non-metrical phenomena. And in and through all scientific methodology there is the assumption of metrical quality as primary and ultimate. The method, as I have outlined it, holds in biology as in the physical and mental sciences and is, in fact, the intellectual formulation of that approach to phenomenal reality which we call science; the approach through inductive logic.

The scientific method is undoubtedly the most useful and successful tool the human mind has ever invented. Science adopted the pragmatic criterion of truth, namely successful control, and as a result science has been successful. It has proved a perfect instrument for research and the modern world is largely the result of its application. In dealing with inanimate things its success has been so overwhelming that we have accepted it almost without question, the only doubt in men's minds being whether the human race can continue to control the weapon it has forged, or whether Samuel Butler's phantasy may come true. It has given to men's minds the idea of a strictly causal and universally ordered nature, with the corollary of ultimate understanding and final classification.

The successful application of the method to inanimate nature encouraged biologists to apply it to animate nature, again with success, although, as yet, this success is not comparable with that attained by students of the inanimate realm. So far as the stuff and structure of living things are concerned, its success has been impressive. But living things are not merely stuff and structure; throughout all life and characteristic and distinctive of life there is "purposive" or organised activity. I have an aversion to using the words "purpose" and "purposive," but I know of no others to express my meaning. I use them here with no implication of consciousness or even of explanation, but purely in the sense of teleological description, that is, of internally determined and directional process deriving from the concept of organisation, such, for example, as the representation of elements in a process in terms of the direction and end of the process, or of maturity in terms of the stages and relationships of development. I am not advocating any interventional teleology or substantial or ontological vitalism, which seem to me as inadequate as any methodological or biological mechanism, but merely stating that, to me, the concept of teleology, or the concept of organic hierarchical organisation in the purely descriptive sense, is an essential element in the unity pattern by means of which life can be described and understood. This organismal concept seems to me, as yet, inherently irreducible to the conceptual plane of chemistry and physics although, of course, this does not in any degree rule out the occurrence of ateleological events or the use of mechanistic explanations and hypotheses as most valuable and entirely legitimate tools in biological enquiry. The teleological or organismic approach, or a methodology based upon the gestalt concept has, I believe, fundamental heuristic value in biology, although, at present, the pragmatic attitude still largely remains the most useful one in research practice. When actually investigating, one is conscious of method rather than philosophy. It seems to me quite clear, however, that any theoretical biology must go beyond pragmatism and interpret organismically on a conceptual plane. To close one's eyes to the teleological or organismic nature of living activity seems to me not only to be blind eyed but to be blind minded, to conceive organisms as fantastic abstractions and to lose all sense of realism.

Yet, in the application of the scientific method to living organisms, this "purposive" aspect is ruled out, for "purpose" is not yet a scientific notion. Biologists have made the vital error of equating causal explanation with mechanistic causation and so ruling out purposive interpretation as non-causal. Its elimination means, simply, that the most obvious and fundamental problems of biology are not even approached, since sensory experience of living things, which is the only way we have of knowing them, or immediate experience of ourselves, shows clearly that any complete representation of life must be teleological; the concept organism must comprise the concept organisation, as including internally determined activities. The scientific method has been successful only within a restricted field of biological reality, largely, I think, because we have assumed that the more fundamental factors will always lend themselves for discrimination with peculiar clarity. In thus making clearness and distinctiveness the test of scientific importance we have misapprehended the scientific status of appearance. The human intelligence has enormous limitations and is largely an agent of simplification; for this reason appearance is an incredibly simplified edition of reality.

Further, the intellectual framework of man's thinking about himself is still essentially prescientific. The scientific method has been applied to his stuff and structure with some success, but to his functional activities with considerably less success, whilst we still hesitate to apply scientific method to his "purposive" activities, to his mind and such products of his mind as religion and morals, beauty, truth, art and music. Even smell largely eludes the scientific method. The orthodox experimental psychologists have confined themselves severely to the metrical and conscious aspects of mind and have thrown singularly little light upon mentality. The clinical psychologists led by Freud seem to me some of the very few workers who have approached the mind in a really scientific spirit, and the antagonism they have aroused is a measure of our prescientific attitude towards ourselves.

In the physical sciences, the scientific method has been universally applied and its success has been general; in the biological sciences it has been partially applied and, within a restricted field, has been successful. If we, as biologists, believe that the scientific method is of universal validity, then we must have the courage of our faith—and it is a faith—and be prepared to apply it to all aspects of living phenomena. At present, by restricting our vision, we select our phenomena to fit our method and close our minds to such phenomena as do not fit. But, if we believe the scientific method to have universal validity, then there can be no reserved and mandated territories in natural phenomena. If, on the other hand, we refuse to apply the scientific method to *every* natural phenomenon, then we must admit that the method has only partial validity and, in that case, may only be one of many approaches to reality and, at best, can only give us a very partial and distorted view of the universe.

Let me, for a few moments, consider the structure and implications of the scientific method, since our use of the method and our mental conception of it are the foundation of all our work.

We say that we observe facts, but these facts are merely instances, and they are events and not things. Further, since all data are mental events, the qualities we observe are in us and we merely externalise them. The phenomena we sense are not dictated by the impartial facts, for our sensitive mind selects and discards, and what it retains is rearranged in a subjective order of prominence. Much of what we call reality is merely subjective appearance, and perception is shot through and through with recognition and projection: phenomenalistic theories of perception seem to me entirely inadequate.

In describing phenomena we remove ourselves a step further, since we describe interpretation and not stark sensa. Sense perception is always accompanied by interpretation and the mere use of words involves inferences which may be mistaken. In fact if we analyse this process, less and less is found to be brute datum and more and more is found to be inference. The inference is, of course, unconscious but is not thereby necessarily valid. Observational order is always interpreted in terms of the concepts supplied by the conceptual order; the two interplay, new observations modifying the conceptual order and new concepts suggesting new possibilities of observational discrimination.

In forming working hypotheses we reconstruct by inference, and very partially, our minds guided by prejudice and belief along lines predetermined by the general intellectual climate of our time. Moreover, the actual process is usually not inductive but deductive, the hypothesis coming before and not after the data, and thus rendering possible the appreciation and arrangement of the data which exemplify it. Purely subjective factors are fundamental in the creation of scientific theories, and our attitude towards theories depends on considerations which are, at bottom, affective. A kind of reasoning which will convince one type will not convince another, and these psychological differences are fundamental, and play a great part in the actual construction of science: science and art are closely akin.

Proof is merely a matter of increasing the degree of probability, and the testing is according to an experimental technique guided and restricted in every direction by mental and physical impulsions and barriers, with every observed and described fact subject to such inhibition, selection and prejudgment as I have indicated. The experimental confirmation is thus a partial and selective testing of a selected and partial hypothesis of doubtful induction framed by interpretation of inferences drawn from selected and partial data. It is one of the greatest thinkers of our day who has declared that "experiment is nothing else than a mode of cooking the facts for the sake of exemplifying the law."

And, finally, our scientific laws are merely convenient and temporary approximate statements with limited ranges of validity.

Further, it must be recognised in all scientific work, not only that method is largely dictated by theory but that the relevance and pertinency of evidence derives, to a considerable extent, from the theory which, often unconsciously, is dominating one's mind at the time of any particular consideration. To attribute supreme value in our scientific thought processes to logical coherence and clarity is to show a complete lack of understanding of the way in which the human intelligence functions. We do not arrive at scientific conclusions by the respectable process of quiet logical deduction nor yet by the blinding flash of intuition, but by a shoddy untidy process something between the two. Our reasonings are blind gropings clutching at threads for premises and floating on thistledown for deductions.

Although, at every step, the relata are given objective reference, yet such objectivity is itself largely conceptual and, with each step in the scientific procedure, the whole process becomes increasingly abstract and remote from phenomenal reality. In physics, which is the most advanced science to-day, this remoteness and abstraction have become so extreme that many fundamental concepts which biologists naïvely assume, have been criticised so destructively as no longer to be seriously considered; much of our tacitly accepted biological methodology is on the physical scrap heap; basic principles such as monism, continuity, causality and determinism are open questions, classical laws have become mere conventions as to measurement, and our universe and all the living things we study, and we ourselves, have been reduced to a mere "wave of probability undulating in nothingness." On the one hand intellectual abstraction in physics has carried the science successfully to heights scarcely within the contemplative vision of the biologist and, on the other hand, physical theory, by undermining the whole structure of applied reason, has taken our solid workaday world and has presented us with a world of shadows and fantastic dreams.

And the very scientific methodology which has borne and nurtured these things has, so doing, almost destroyed its own being, and physicists are now facing a situation where the scientific frame of thought is too circumscribed and rigid for the concrete facts which are before us for analysis. It is in physical science that the philosophical validity of the scientific method is to-day receiving its most destructive criticism, and where the position is openly admitted, that the standardised concepts of science may be valid only within limitations which are, perhaps, too narrow for science itself.

The only rivals to the physicists are the psychologists, many of whom hold that "The question at issue is no longer whether psychology is capable of achieving the necessary standards of science, but whether science is capable of fulfilling the requirements of psychology," and who are wondering whether, in the field of psychology, the method of science may not prove to be "an ill-adapted and essentially inadequate weapon."

The position in biology seems to me clear. We have accepted the validity of scientific methodology without question; we have not even thought it possible to question it, and we have compromised by tacitly restricting the field of our subject to such an area as the scientific method fits and then denying the existence of the excluded regions or brushing them aside as "mere teleology." The primary concepts of biology seem to me clearly inadequate to our most important problems, the really important facts of nature eluding the scientific method. For example, it is surely the most obvious fact about organisms that they are organised and that the organisation is hierarchical and teleological; yet these facts are eliminated from most biological research to-day and are very difficult to fit into the frame of modern biological theory. Further, most of our biology deals with organisms in their space dimensions and almost entirely neglects their equally obvious and fundamental time dimensions, which leads to the perceptual but inherently unreal antitheses between function and structure, phylogeny and physiology, or epigenesis and preformation. Instead of building new concepts and methods we eliminate the problems and pretend the facts before us do not exist. The issue facing us is not how we can select and arrange our data to fit into our science, but how we can build a science to fit the data.

Now, in pure biology and within its tacit boundaries, the scientific method has worked extraordinarily well and its pragmatic success is exemplified in the vast and rather wonderful descriptive structure of our science. Pure biology is very largely a laboratory and museum study in which the subject is isolated from its natural habitat and investigated under controlled conditions. It is the study of "single phenomena divorced from the community of life." That is to say, we apply the scientific method to selected material in accordance with a selective problem, and so necessarily study such phenomena as are susceptible to the method. On the whole, therefore, the pure biologist will find little cause to question his method.

The applied biologist who cannot select a theoretical problem, who cannot in the same degree isolate and control his material, but who must face the practical issue as it exists in all its complexity in nature, is much more likely to find the scientific method inadequate and to wonder whether it may not possess only restricted validity. I do not here mean mere differences in points of view, important as these are, for example, the species concept of the systematist, the geneticist and the pathologist. I mean the kind of thing that I am faced with in my own research on *Botrytis cinerea* and its welter of races, and I may perhaps instance two of many issues confronting me.

From long, perhaps unduly long, experience of these races I can recognise—"intuitively" if you like—differences which I can neither measure nor describe; I simply know them because of a heightened sensitivity to them developed by experience. And, knowing them in this "intuitive" way, I can appreciate relations which are meaningful for me but for which I cannot adduce such data as would convince another *without my experience*. The whole thing is conceptual rather than perceptual. I am a little comforted in my unease when I find Einstein saying: "There is no logical way to the discovery of these elemental laws. There is only the way of intuition, which is helped by a feeling for the order lying behind the appearance, and this *Einfühlung* is developed by experience." The difficulty is to sift the gold of a real intuition from the dross of personal predilections and prejudices. My difficulty may ultimately be one of technique only, although I can, as yet, see no way to a solution. My efforts merely seem to reveal the apparently insoluble nature of the problem and the fact that, if I crystallise the problem into a particular setting, when I can deal with it, I am entirely falsifying the true problem in order to make it fit my method. It is rather like taking a single photograph from a cinema-film, if one can imagine a multi-dimensional cinema-film in space-time, and assuming that the single photograph is a portrayal of the events and endurances recorded in the film. One cannot too clearly realise that an organism never *is* at any one moment, it is always *becoming*.

A certain amount of confusion is, I think, introduced by the idea, tacitly assumed, that all science is metrical, that the real may be identified with the quantitative, but it seems to me quite clear that, in biology, much of the recording and augmentation of our experiences, which is essentially scientific, is not metrical. Mathematical relations are external to the terms they relate whereas organic relations are internal to their terms. In biology, therefore, we need to employ a unity pattern of a different kind, a unity of differences and not of identities.

Further, I think that in biology we may be faced with the situation now existing in mathematical physics, where essentially non-verifiable hypotheses constitute a new instrument for scientific correlation, or where mutually exclusive hypotheses may both be pragmatically true. A hypothesis may contain real truth and phenomena may be just useful tools. Further, we must not postulate simplicity, for it is becoming ever clearer that simple solutions are sham solutions, the stubborn irreducible facts being often merely irrelevant incidents obscuring the more fundamental and significant processes in the organism. Intelligibility has nothing to do with truth and the elements of experience are not necessarily true and important in proportion to their clarity in our consciousness or our vision.

My second illustration arises in this, that I have come seriously to wonder whether the *Botrytis cinerea* complex as it exists in nature is, in any sense, orderly or rational. Growing in regiments of bottles in the laboratory there is order, too much order, since at any particular time a particular order is merely a question of a particular measuring stick. Even then there is a sort of, shall I say "Heisenberg uncertainty" about things, a certain freedom which may be only accessible to a statistical treatment in qualities and characters which are not yet statistically accessible. But the concrete problem is not *Botrytis cinerea* in regiments of bottles under standardised conditions in the laboratory at a particular moment but *Botrytis cinerea* on mobs of plants in the open under all sorts of varying conditions in time.

This immediate problem alone raises in my mind the whole question of the conception of an orderly universe. Is the universe orderly *per se*, or is order a humanly imposed condition created by selection and restriction; is it merely due to our passion for pigeon-holes? All our science is based on the faith that nature is orderly and that the scientific approach to natural phenomena will give us a true picture of reality.

It is usually accepted that science approaches perfection in the degree to which it becomes mathematical, but, as I have said, I can see no *a priori* reason to suppose that the universe must be the kind of thing that is innately orderly and can be described mathematically. In my own work and thought the more I pursue precision the further I leave certainty behind. I think a realisation of this is peculiarly important at the present time, when there is an increasing and rather intolerant tendency to force biology into the frame of mathematics. The part played by measurement and quantity is very great but it can, I think, be overestimated. Mathematical technique is a splendid tool and should be applied wherever possible but, to hold it as an article of faith that all nature is necessarily mathematical is to cling to an obsolete metaphysics and to assume, in the face of all immediate experience, that the physical unity pattern is the organic unity pattern. It should be clear that a law may be quite scientific without being quantitative, and biology must be free to be influenced but not controlled by mathematics; mathematics is a critic and not a guide.

A belief in universal order does not, in any way, impose itself on my mind as an outcome of direct experience. One's intellect seizes in the reality the element we call order, but it does not thereby encompass reality, and much of our science seems to me merely a device for imposing order upon sense experience. Science assumes that nature is a rational whole, but there is no law to the effect that things that seem odd to us must not happen, or that what we find convenient assumptions in science are necessarily true although, on the whole, their pragmatic success in giving control over external nature has been stupendous.

During the last quarter of a century the physical sciences have made almost fabulous progress, and have not only revolutionised thought but have revolutionised man's very way of thinking. This has been done by driving scientific methodology to its uttermost limit, by largely abandoning empiricism and spatial imagery and by a startling use of imaginative speculation guided by intuition.

On the other hand, biologists have been so obsessed by the vast panorama and infinite variety of life which has opened up to their vision that they have not driven the scientific method hard or made any enquiries into its metaphysical foundations; they have been content with scientific empiricism and they have eschewed speculation. Biologists seem to have an innate aversion for thinking theoretically and still cling to the Baconian notion that, with a sufficient care in the collection and description of instances, the general law will stand out by itself; although any reading of modern physical and mental science, or even biological science itself, will show that this is a totally inadequate account of the processes which issue in scientific generalisations. There has been very little genuine induction in biology, with the result that the science is in grave danger of being smothered in its own promiscuous accumulations of data.

The consequence of this positivist attitude is that in biology, apart from such things as the cell theory, the theory of natural selection and the theory of chromosome inheritance, we have practically nothing corresponding with physical theory. Even these biological theories are formulations along acknowledged lines and are not the intellectual conquests, the revolutions in thought and fundamental ways of thinking which characterise physical theory. In fact the most striking thing in biology compared with the physical and mental sciences is the almost complete absence of theory: theoretically we are where the physical and mental sciences stood a generation ago. To my mind the overwhelming need in our science to-day is the development of a theoretical biology: empirical knowledge, however extended, does not suffice for the foundation of a well-systematised science.

Further, the newer ideas in physics have as yet hardly impinged on biology, although it is quite clear that they must revolutionise our philosophy and attitude to nature, since living organisms are in part physico-chemical systems and life must have space-time relationships. Scientific explanations are little more than a reflection of the intellectual climate of our time, and our intellectual climate is being fundamentally changed by physical thought. Our biological thinking is still profoundly coloured by Newtonian concepts and assumptions which have been found insufficient or have been discarded in physics and the mental sciences but which remain unquestioned in our biological philosophy.

Now speaking quite personally, I believe that in biology we are on the verge of a development of theory corresponding with that which has occurred in the mental and physical sciences, and which will not only change our views of life and of living things but will change the fundamental technique of our everyday thinking about these things. It seems to me probable that this development will come about in biology by very much the same processes that have taken place in the physical and mental sciences, namely, by a driving of scientific methodology to its uttermost, the considerable abandonment of empiricism, the bold use of intuitive and imaginative speculation and the philosophical criticism of biological foundations in the light of modern epistemology.

The collapse of nineteenth-century dogmatism shows clearly that the scientific disciplines, and biology in particular, demand that our imaginations reach out to possibilities beyond our present scientific explanations and that our intellectual horizon be widened to include sensa beyond our present biological data. New directions of thought are rarely born of systematisations of logic but arise from flashes of intuition in the joyous adventure of inspired speculation. And it is such intuition which brings new material within the scope of scholarly learning. Further, all our present biological explanation is in terms of pictorial and spatial imagery, and I think we shall need to abandon this and accustom ourselves to purely conceptual explanation.

I believe that applied biology has a vital part to play in these impending developments, partly by providing an enormous body of data as material to work upon, partly by reason of the existence in applied biology of a large number of research men in intimate contact with field conditions, competent to test the new developments in a natural setting, and partly in the influence which applied biology will have in keeping the concrete problem ever before the speculative vision. Further, I think that the applied biologist may actually lead the van in this progress, being more liberated from traditional viewpoints and conservative ways of thinking than the pure biologist. The timidity and abstraction of academic scholarship tend to circumscribe reason by reducing its topics to mere sensa and prolixities, and "for its refreshment imagination must return to naïve experience" and the practical issues of daily life.

In conclusion. It is often overlooked that all human life and, consequently, all human thought, depends in the last resort upon agriculture, which is largely applied biology. If the power to see things in their right perspective be a primary trait in the cultured mind, applied biology must increasingly assume a dominating importance in our stream of culture. Social ideals and Utopias are fantastic dreams unless apprehended in a perspective of applied biology, and even a little biological wisdom in modern statecraft could ameliorate, if not solve, many of those problems which threaten to swamp civilisation in our world to-day. It should be very clear to everyone that biology, in the larger and more humane sense in which I have tried to discuss it in this address, may help to make the human race capable of a real civilisation, and that political or economic creeds and social or religious sentiments, acting in ignorance of such biological knowledge, may be dangerous forces, capable of producing devastating results in the world. Mankind is still a prey to intolerant reactionaries and intolerable visionaries.

So far as I can see, looking at the great fields of research where significant developments are being initiated, the most fundamental issues of the coming generation will be regarding things of life and things of mind; not merely things of matter. The last generation has been one of physical and chemical exploitation of the world but, in the development of the world of to-morrow, the overshadowing events will be the exploitation of biological and psychological knowledge, that is, the exploitation of living things and mental potentialities. When we are dealing with inanimate things it is comparatively easy to build a new social structure and a new philosophy of life to fit the new knowledge and changing conditions, although mankind seems to have found the task difficult enough. But when we are faced, as we shall be faced, with the building of a new social structure and a new philosophy of life to fit a world in which plants and animals, and man himself, are exploited as were the things of physics and chemistry during the last generation, and not only men's bodies, but those far more private possessions, men's minds and personalities, their beliefs and their hopes, then the task will be one of supreme difficulty. It will demand individual reorientation and social reorientation; no man will be able to stand aloof, and men will participate and build truly in proportion to their biological knowledge and vision. Applied biologists will need to think theoretically and will need the courage of their convictions for, in the building, they must play a leading rôle.

GRASSLAND EXPERIMENTS IN MID-WALES; THE CILMERY PARK EXPERIMENTS

By WILLIAM DAVIES, M.Sc.
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(With Plate XIX.)

In Great Britain there has been a tendency in the past to concentrate our efforts in pasture improvement upon areas at low elevations—areas normally subject to arable rotation of one form or another. Pastures of this type form an integral part of our agriculture, but their importance does not in any way lessen the need for an improvement in those extensive areas of rough grazings that are to be found all over Britain. Rough grazings are by no means limited to the very poor soils. They include those areas of rush-infested land which are all too common on valley bottom soils—soils potentially capable of carrying a good class of pasture. It has recently been shown (1) that the area of rough grazings in Britain has increased steadily during the post-war period. It is probably being added to during the present period of depressed prices.

We already have a body of evidence which shows that very simple seeds mixtures containing a basis of appropriate strains of perennial rye grass and white clover are eminently suitable for producing permanent grass, and further, that cocksfoot, crested dogstail, rough-stalked meadow grass, red clover, Italian rye grass and certain other species each have their special place to fulfil special purposes. The basis for such mixtures is, however, to be found in pasture strains of perennial rye grass and white clover. Productive leys containing these species make considerable demands upon soil fertility. On most soil types they can be maintained at a high level of efficiency, only if given periodic and liberal dressings of fertiliser to replenish the normal drain made upon the mineral and other plant foods in the soil.

The experimental work reviewed in the present paper is concerned chiefly with the improvement of the better class of rough grazings, extensive areas of which occur in western Britain. The work has been conducted at Cilmery Park, in Breconshire on the estate of Mr S. M. Bligh. The economic aspects of such improvements as have been attained are discussed by Bligh and Prewett (2), while the earlier botanical results have been reported by Stapledon and Davies (3).

The Cilmery Estate lies in a zone of high rainfall between Builth Wells and Llangammarch, at an elevation ranging from 450 to 1000 ft. The average rainfall is approximately 60 in. per annum. The natural forest vegetation of the district is of three main types, namely, (1) the oak woodlands of the valleys; (2) the well-developed birch woods of the slopes (up to 700 ft. elevation); and (3) an open, stunted and sparse birch scrub with gorse and bracken on the poorer hillsides and at elevations between 700 and 1000 ft.

The type of land under discussion has been described in a previous publication (3). Broadly speaking, the fields in their unimproved condition, namely, the condition in which they were to be found at the outbreak of the Great War, fall into the following categories, based on the vegetation which they carried in 1915, and on notes supplied by Mr Bligh:

(1) Bracken and gorse brakes.

(2) Birchwoods with patches of rough grass, bracken and rushes.

(3) Heath vegetation: (*a*) dry fescue-*Agrostis* pastures and (*b*) wet *Molinia-Nardus* with sedges and rushes.

(4) Fair average pastures of the *Agrostis*-white clover type with perennial rye grass, dogstail and a few other valuable pasture plants.

The latter category occupied in 1915 only a small proportion of the total area on the estate, but, however, carried the bulk of the then grazing stock. The rough pastures and brakes placed in the first three categories were characteristically non-leguminous and were of negligible grazing value. In general, these areas had not been under the plough during the period between the Napoleonic Wars of the early nineteenth century, and the Great War of 1914. Subsequent to 1915, fairly detailed records have been kept and a comprehensive series of regrassing trials has been conducted on the estate. The greater part of the birch, gorse and bracken is now cleared and a large proportion has been ploughed and regrassed. The majority of the better fields have been laid down under oats without introducing a crop rotation. Occasionally a short rotation consisting of oats, roots, oats, grass has been followed. Where fields were too wet or too rough to be ploughed, other means of mechanical treatment have had to be evolved (5).

None of the soils at Cilmery can be regarded as naturally fertile, although adequate dressings of phosphatic manures have brought about a marked improvement in their fertility. Particularly is this true where applications of phosphates have encouraged the prolific growth of wild white clover. The problem of increasing the general standard of fertility on such soils is fairly straightforward, but some difficulties have been experienced where the vigorous development of clover is at best a slow process. The interesting studies being conducted by Poulter (4) concerning inoculation by specific bacterial organisms have an important bearing upon the problem of clover spread on the hill soils. Inoculation is shown to have an important effect upon the development of white clover on some of the dry slopes of low fertility at Cilmery. It is evident from the results already reported upon by Poulter that the scarcity of requisite noduliferous bacteria can be very marked on those pasture types which in the unimproved state are characterised by a deficiency or even by a complete absence of leguminous plants.

The Cilmery work has led to a wider appreciation of the grassland problem in Wales, particularly that concerning rough grazings. Whereas in the Cilmery trials the plough is freely used as a grassland implement, in a number of instances it has not been possible to plough because of the unevenness of the ground and the damage to ploughs from old tree stumps. The expense of levelling and logging would be prohibitive. The result is that other forms of mechanical treatment have been evolved in order to deal with these particular areas. Some form of roughly made implement has been dragged over them by means of a tractor. These implements have been mainly home constructed by farm labour. An essential characteristic in each is that when drawn over the surface of rough grassland, the "mat[1]" is broken and a proportion of the underlying soil is exposed. In addition, ant-hills, mole-humps and other excrescences are levelled off. The greater the proportion of "mat" which is torn away, the greater is the efficiency of the mechanical treatment. Mr Bligh has attained marked success in this direction with heavy logs—usually trunks of trees—tied together with wire hawser (of the type used for ships' rigging). These are drawn behind a Fordson tractor and very effectively level down the ground as well as tear up the "mat."

Whether the field is ploughed or surface cultivated the essential part of the procedure is that as much as possible of the rough herbage is destroyed. Subsequent to this a seeds mixture is sown, wild white clover taking a prominent part in such a sowing. On a number of the rough fields that have been "scratched" over, the seeds mixture has consisted of seedsmen's cleanings (5) out of old pasture seeds. Such cleanings are only purchased when guaranteed to be from old pastures, and when the sample shows a good proportion of wild white clover, perennial rye grass and dogstail. In addition fog and wild red clover have, in certain cases, supplemented the mixture. It cannot be too strongly insisted upon that such cleanings should emanate from old pastures; in comparison with this the relative germination, purity and weed content is of but secondary importance. It might also be a good plan always to supplement the cleanings with, say, 1 lb. of wild white clover plus 4–5 lb. of a good pasture type of perennial rye grass to the acre. This would be an insurance against any marked deficiency in the cleanings which might otherwise be overlooked, and at the same time would not make the mixture unduly expensive.

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[1] Undecomposed and semi-decomposed herbage characteristic of rough grazings in Wales.

It should again be emphasised that the natural pastures at Cilmery were almost completely non-leguminous. The first precept in any scheme of pasture improvement, therefore, is the successful introduction of an efficient pasture legume. In Great Britain wild white clover is the legume *par excellence* for this purpose and therefore the first sets of operations must have concern for the successful establishment of white clover. It has repeatedly been the experience that once the sward has been through a phase approaching to clover dominance it is easy to follow up with a pasture in which high fertility-demanding grasses will take a prominent, if not a dominant, place.

At Cilmery it has been found fairly easy to transform the outrun grazings on the better class of soils into clover-dominant pastures. Sowings of wild white clover, together with liberal phosphating, has made this feasible. Furthermore, persistent strains of perennial rye grass and cocksfoot (as exampled by pedigree lines developed at Aberystwyth) make it possible to maintain these swards at a high standard of botanical composition with perennial rye grass, cocksfoot, dogstail and white clover as the dominant constituents.

Discussion of botanical evidence.

The several fields, the botanical evidence from which will be discussed, are enumerated below; a note is added in regard to the vegetation type existing on them in 1915.

"C" Ground: gorse, bracken and thick birch. Used as a fox covert.

Comyn Fields: in 1915 a bent-fescue pasture with bracken and a large number of ant hills. A useful cover for rabbits.

Spring Field (*Coryn*): a rotation field probably left down 5–8 years to grass, followed by the crop rotation typical of the district.

Lower Tyreinon Field: bent-fescue with much bracken. Maintained as a rabbit cover.

Deral Field: heath-fescue pasture. Dominant sheep's fescue with bracken.

Molinia *Field* (*Gwarafog*): wet land infested with tree roots and stumps; neglected pasture *Molinia* dominant, with sedges, rushes, *Nardus*, etc.

Warren and Ectroma Fields: damp land, birch-alder scrub with ground flora rushes. Bracken on drier knolls.

Table I.

"*C*" *ground. Summarised botanical evidence on plots sown* 1924 *with and without indigenous rye grass and cocksfoot. Analysed ninth harvest year* (25. v. 33). *Estimated percentage frequency tillers.* E. 52. VII.

Species	Percentages		
	With indigenous perennial rye grass (plots 5 and 7) (1)	With indigenous cocksfoot (plot 3) (2)	Without indigenous strains of either perennial rye grass or cocksfoot (all other plots) (3)
Perennial rye grass	25	—	2
Cocksfoot	—	10	Traces
Rough-stalked meadow grass	8	10	5
Tall fescue	Traces	—	—
White clover	30	30	28
Bent (*Agrostis*)	22	40	43
Yorkshire fog	8	10	10
Other grasses (not sown)	2	Traces	4
Miscellaneous plants	5	Traces	8
Total	100	100	100
Summary:			
Sown grasses	33 } 63	20 } 50	7 } 35
White clover	30	30	28
Other plants	37	50	65

(1) *The "C" ground* (E. 52).

Table I shows the estimated percentage frequency[1] in the ninth harvest year. It is of interest to compare the plots where indigenous perennial rye grass and indigenous cocksfoot respectively were sown. It will be seen that rye grass still contributes 25 per cent. to the sward

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[1] All botanical data given in this paper have been obtained by methods described by Davies (7).

(column 1), while cocksfoot is found to the extent of only 10 per cent. (column 2). It is interesting also to observe that rye grass has been better able to keep out bent and fog than has cocksfoot. A comparison of these data with those in column 3 is significant, particularly as regards the persistency of commercial and pedigree rye grass and cocksfoot. The commercial strains of these two species have practically disappeared and in any case are making but negligible contribution to the ninth year sward (column 3). Significant, too, is the fact that while those commercial strains have failed to persist their place in the sward has been taken by bent (*Agrostis*). The white clover content does not at this stage (ninth harvest year) vary significantly on any of the plots, but while it might be expected that either the sown wild white clover or the volunteering local strain would fill in the gaps caused by the disappearance of the commercial rye grass and cocksfoot, this has not in fact taken place. Bent which is propense to the district, and which has been relatively encouraged by understocking in summer, has proved altogether too aggressive and has dominated the pasture to the detriment of white clover.

In a previous communication (3) it was shown that bent and fog may be regarded as "followers of man" on the Cilmery grasslands. Fine-leaved fescue enters largely into the composition of these pastures, but after being ploughed, phosphated and sown with a commercial seeds mixture, the succession is towards a dominance of bent and fog with a marked diminution of fine-leaved fescue. It is now clear, however, that if pasture strains of perennial rye grass, cocksfoot and white clover are included in the seeds mixture, bent is at least kept under control. At Cilmery the pastures are almost invariably understocked during the summer, while the grazing is relatively severe in the early spring. This factor alone would tend to depress the growth of perennial rye grass and of wild white clover, while assisting considerably the spread of bent which makes its maximum growth in summer and is late to start active growth in the spring. Even so, the results show very clearly that perennial rye grass of an approved pasture strain is able to combat the aggressive bent and at least to relegate the latter to a subordinate position in the sward. Commercial strains of perennial rye grass have not persisted and bent has thus become the main sward aggressor.

Tall fescue (using an indigenous type) has made practically no contribution to the ninth year sward. Earlier trials at the Welsh Plant Breeding Station have shown that tall fescue does not normally establish itself well in the field unless sown in large amount and as the predominant element in a simple mixture. When sown in a mixture with rye grasses, as was the case on the field under discussion, its establishment is considerably depressed, often to a point at which it may be said not to have become established at all.

Table II.

Comyn Fields (E. 68. I). *Summarised botanical evidence on plots sown* 1925 *with and without indigenous perennial rye grass. Analysed eighth harvest year* (26. v. 33). *Estimated percentage frequency tillers.*

Figures in brackets indicate the range of seed rates in lb. per acre on the different plots.

Species	Percentage	
	With indigenous perennial rye grass (plots 3–6)	Without perennial rye grass (plots 1, 2, 7 and 8)
Perennial rye grass	30 (7–14)	— (—)
Italian rye grass	— (10)	— (10–15)
Rough-stalked meadow grass	13 (0–3)	18 (0–6)
Crested dogstail	5 (0–4)	— (—)
White clover	22 (3–4)	22 (3–4)
Bent (*Agrostis*)	15	35
Yorkshire fog	10	10
Other plants (unsown)	5	15
Total	100	100
Summary:		
Sown grasses	48 } 70	18 } 40
White clover	22	22
Unsown species	30	60

(2) *The Comyn Fields* (E. 68. I), *sown* 1925.

The data shown in Table II refer to plots in their eighth harvest year, and are a further indication of the value of pasture strains of perennial rye grass in sward maintenance. It will be seen that perennial rye grass with wild white clover is a pivotal species, particularly as concerns the proportion of unsown plants. Plots sown with—as opposed to those without—perennial rye grass have a much lower bent content, the figures being 15 and 35 per cent. bent respectively. A similar trend is noted in the case of miscellaneous plants and weeds, but the proportion of fog stands at 10 per cent. in both sets of plots. The lower figure for rough-stalked meadow grass on the rye grass plot is attributable to the greater density of sward. The presence of rye grass, however, proved no more detrimental to white clover than the presence of an undue proportion of bent. The figure for white clover is similar on both plots. The botanical evidence from these Comyn fields confirms in all essential respects that from the "C" ground discussed above. The further comments arising from the study of the detailed evidence upon which Table II is based are as follows:

(*a*) There is no discernible difference in the amount of white clover present on plots sown with this species at the rate of 4 and 3 lb. per acre respectively.

(*b*) Plots 3 and 4 seeded with perennial rye grass at the rate of 7 lb. per acre show 25 per cent. of this grass, while on plots 5 and 6, seeded at the rate of 14 lb. per acre, the rye grass contributed 35 per cent. of the sward (eighth harvest year).

(*c*) On the damper habitat represented by lower Comyn Field, rough-stalked meadow grass made an unsown appearance of 20 per cent.; while on the drier upper Comyn a seeding of 6 lb. per acre (mixtures 7 and 8) contributed only 17 per cent. to the herbage of the eighth harvest year. In both sets of mixtures Italian rye grass and wild white clover were used as the other components, rough-stalked meadow grass being included in mixtures 7 and 8.

Table III.

Spring Field (E. 68. II). *Botanical analyses of the plots sown* 1926. *Analysed fifth harvest year* (1931). *Estimated percentage frequency tillers.*

Figures in brackets denote seeds mixture in lb. per acre.

	Percentage				
Species	Plot 11	Plot 12	Plot 13	Plot 14	Average all plots
Perennial rye grass (indigenous)	37 (5)	52 (10)	56 (10)	49 (14)	48·5
Italian rye grass	— (3)	— (3)	— (3)	— (3)	—
Rough-stalked meadow grass	23 (10)	31 (5)	17 (3)	16 (3)	22·0
Crested dogstail	11 (10)	4 (—)	8 (3)	9 (3)	8·0
White clover	19 (4)	9 (4)	5 (3)	12 (3)	11·0
Other grasses*	8	2	13	11	8·5
Miscellaneous plants	2	2	1	3	2·0
Total	100	100	100	100	100·0
Summary:					
Sown grasses	71 } 90	83 } 92	81 } 86	74 } 86	
White clover	19	9	5	12	
Unsown species	10	8	14	14	

* Mainly fog and bent.

(3) *Spring Field Coryn* (E. 68. II), *sown* 1926.

This field is peculiar among those discussed in this paper in that it had been through a complete rotation including a root-cleaning crop prior to being laid down to pasture. The field as a whole has given remarkably good grazing since it was laid down in 1926. It stands at an elevation of about 700 ft. and the land cannot be regarded as naturally fertile even within the district concerned. It was in good heart, however, when laid down to grass. The data given in Table III concern the fifth harvest year. Each seeds mixture contained indigenous perennial rye grass in amounts varying from 5 to 14 lb. per acre. Italian rye grass at 3 lb. per acre was sown in all plots. The seed rate of dogstail varied from nil to 10 lb. per acre, of rough-stalked meadow grass from 3 to 10 lb. per acre, and of wild white clover from 3 to 4 lb. per acre in the different mixtures. All the seeds mixtures were in essence very simple and represent the standard ultra-simple mixtures advocated by the Welsh Plant Breeding Station for pasture leys. The mixtures were designed to test sward reactions consequent upon varying the balance of species within such a simple mixture.

The standard of botanical composition maintained into the fifth harvest year is a high one, as measured by the proportion of sown species still persisting. Compare the composition of this field (averaging all plots) with the average of the plots containing indigenous rye grass (that is the best plots) from the "C" ground, from Lower Tyreinon (see Table IV) and from the Comyn Fields. The following statement makes this comparison clear:

	"C" ground	Comyn Field	Lower Tyreinon	Spring Field
Harvest year ...	9th	8th	5th	5th
Perennial rye grass	25	30	35	49
Rough-stalked meadow grass	8	13	16	22
Crested dogstail	*	5	5	7
White clover	30	22	20	11
Other species (unsown)	37	30	24†	11

* In this instance dogstail only occurred in small proportion and was included with unsown species.

† Includes 8 per cent. fine-leaved fescue (sown).

It will be seen that of the fifth-year sward on Spring Field, 89 per cent. was still being contributed by sown species, perennial rye grass itself constituting 49 per cent. of the sward. The high proportion of rough-stalked meadow grass is noteworthy as is also the low relative proportion of dogstail. This is in conformity with the general trend of our evidence, namely, that dogstail cannot withstand heavy competition in dense, highly productive pastures, particularly where summer grazing is not severe. Dogstail is likely to prove invaluable on poor soils, but does not show to great advantage under more fertile conditions, such as those met with on the field under discussion. It may be said that where rough-stalked meadow grass makes luscious growth, dogstail is likely to be adversely affected by competition. Conversely, dogstail becomes an extremely useful grass on land where rough-stalked meadow grass is stunted and unproductive. These two grasses, therefore, would seem to be complementary in their agronomic values on contrasting classes of soil. The figure of 11 per cent. (Spring Field) given for wild white clover is low in comparison with the figures for the other fields. This is largely due to the productiveness and density of the sward as a whole, accentuated by the fact that the summer grazing at Cilmery is always insufficiently heavy. On fields which are at a reasonable level of fertility and where in any case white clover makes adequate contribution to the herbage, the actual percentage of white clover at any given time is largely a function of the closeness of grazing during the preceding period. This statement seems to be equally true on pastures in other parts of Britain. For example, on the Midland fatting pastures the proportion of white clover to grasses is low in, and after, wet seasons when growth generally is superabundant. Following upon periods of close grazing, on the other hand, such as occur during dry seasons, the ratio of clover to grass shows a corresponding increase. The fluctuations of the clover-grass ratio in permanent pastures is well recognised in practice as is suggested by the terms "good white clover years" and "poor white clover years." It is doubtful if a superdominance of white clover is required on good-class lowland pastures. Rather should the clover be subdominant to perennial rye grass. What really matters is that white clover should be present in telling amount and that the grazing technique should be such that its place in the sward and its healthy condition are assured from year to year.

Referring to the detailed data presented in Table III it is seen that while a seeding of 5 lb. per acre of perennial rye grass has provided a 37 per cent. contribution to the sward (mixture 11) larger amounts (up to 14 lb. per acre) have contributed from 49 to 56 per cent. The ultimate proportion of rye grass does not, however, bear any exact relationship to the seed rate, but there is a suggestion that a seeding of 5 lb. per acre is too low a figure.

The different seed rates of dogstail, rough-stalked meadow grass and white clover have not provided significant results. For example, 10 lb. of dogstail gives a 11 per cent. contribution, while 3 lb. has given from 8 to 9 per cent. Similarly in rough-stalked meadow grass, a 3 lb. seeding has given about 17 per cent., a 5 lb. seeding is shown to give 31 per cent., while the 10 lb. seeding has only given 23 per cent., and that with a light seeding of rye grass. These data show that there is no gain in sowing either of these three species (dogstail, rough-stalked meadow grass and white clover) at rates in excess of about 3 lb. per acre. This is reasonable in that the place of these species in the resultant pasturage should be subdominant and subordinate to perennial rye grass (or some other one major grass). On the other hand, the data suggest that the major species should be sown more liberally and at rates not less than 10 lb. per acre.

Table IV.

Lower Tyreinon (E. 86). *Botanical analyses of plots sown* 1928. *Analysed fifth harvest year* (24. v. 33). *Estimated percentage productivity.*

Figures in brackets denote seeds mixture in lb. per acre.

	Percentage			
Species	Plot 1	Plot 2	Plot 3	Plot 5
Perennial rye grass (indigenous)	36 (14)	— (—)	40 (14)	28 (14)
Italian rye grass	— (6)	— (6)	— (6)	— (6)
Cocksfoot (indigenous)	— (—)	52 (16)	— (—)	— (—)
Rough-stalked meadow grass	20 (4)	4 (4)	27 (4)	— (—)
Crested dogstail	8 (4)	4 (4)	— (—)	8 (4)
Red fescue (indigenous)	— (—)	— (—)	— (—)	24 (10)
Red clover (Montgomery)	— (4)	— (4)	— (—)	— (4)
Wild white clover	16 (3)	10 (3)	23 (4)	20 (3)
Bent (*Agrostis*)	—	—	—	—
Yorkshire fog	9	8	5	8
Miscellaneous plants	11	22	5	12
Total	100	100	100	100
Summary:				
Sown grasses	64 } 80	60 } 70	67 } 90	60 } 80
White clover	16	10	23	20
Unsown species	20	30	10	20

(4) *Lower Tyreinon* (E. 86), *sown* 1928.

Table IV shows the botanical composition of each of four mixtures sown in 1928 on Lower Tyreinon. The figures given in brackets represent the seeds mixture sown in lb. per acre. Indigenous rye grass and indigenous cocksfoot were sown in the quantities indicated in the table. The trial was set out to test the following points:

(*a*) Relative sward-forming ability of indigenous perennial rye grass and indigenous cocksfoot when sown as dominants in the mixture.

(*b*) Relative pasture values of rough-stalked meadow grass, dogstail and pedigree red fescue under semi-lowland conditions.

(*c*) Influence of Montgomery red clover in pasture mixtures.

The following general conclusions may be drawn from the botanical evidence presented (Table IV).

(*a*) Montgomery red clover has not persisted into the fifth harvest year. While this strain of red clover will have produced valuable feed during the earlier years of the ley its inclusion in the mixture has not caused any material unbalancing of the fifth year sward. There is, however, evidence that the red clover has had some influence, particularly upon the percentage of wild white clover which has been slightly lowered, and upon the percentage of unsown plants which show an increase following the dying out of red clover. (Compare plot 1 with red clover at 4 lb. per acre and plot 3 sown without red clover.)

(*b*) Indigenous cocksfoot as opposed to indigenous perennial rye grass has been instrumental in causing the partial suppression of dogstail, rough-stalked meadow grass and white clover. From equal sowings of these three sward-forming species, there is an aggregate contribution of 44 per cent. to the sward (plot 1 sown with perennial rye grass) compared with 18 per cent. in the cocksfoot mixture (plot 2).

The management of the pastures at Cilmery involves heavy spring grazing, together with lenient summer and autumn grazing. This system would naturally favour cocksfoot, while being detrimental to the spread of the bottom grasses and white clover (6). The data indicate once again that indigenous perennial rye grass provides a unique basal pasture grass which even with a not-higher-than-average standard of grazing technique will allow the healthy spread of bottom species growing in association with it. An approved pasture type of perennial rye grass with wild white clover should form the basis of pasture mixtures on a wide variety of soil types. The additional species in any such mixture will vary from soil type to soil type and in general will be selections from the following: dogstail, rough-stalked meadow grass and fine-leaved fescue as bottom grasses; Italian rye grass, cocksfoot, timothy, meadow foxtail and red clover each to fulfil its special purpose.

(5) *Deral Field* (E. 68. I), *sown* 1926.

Five mixtures were sown on this field in 1926 when last seeded down. The analyses given in Table V were made in 1933, and therefore represent seventh harvest year evidence. The seeds mixture used in each plot is appropriately shown in brackets. The chief interest of this table is the relatively high contribution to the sward which is still being made by the sown species other than, of course, Italian rye grass which normally disappears after the second year. The penultimate column in the table shows the average contribution of sown species, plus fog, bent and other unsown species reduced to a percentage basis. The ultimate column shows the comparable data collected in 1925 (see Stapledon and Davies (3)), and interestingly enough these also relate to a seventh harvest year sward resulting from the 1918 sowing. This field lies on a relatively poor slope, the soil inclining to be clayey and retentive of moisture. Prior to 1917 when the field was first ploughed, Deral was described as a typical heath-fescue pasture with some bracken. A crop of rye was harvested in 1918, a seeds mixture containing, among other species, rye grass, cocksfoot, timothy and white clover having been sown in that year. The 1925 analysis shows that none of the grasses included in the first sowing had survived to the seventh harvest year. It should be borne in mind that in 1918 only ordinary commercial seeds were available, with the one exception of wild white clover—significantly the only surviving species of those sown.

Table V.

Deral Field (E. 68. I). *Botanical analyses of plots sown* 1926. *Analysed seventh harvest year* (25. v. 33). *Estimated percentage frequency tillers.*

Figures in brackets denote seeds mixture in lb. per acre.

	Percentage					1933 relative average %	1925 data 7th year*
Species	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5		
Perennial rye grass (indigenous)	25 (5)	20 (5)	35 (5)	35 (14)	35 (14)	27	—
Italian rye grass	— (3)	— (3)	— (3)	— (3)	— (3)	—	—
Rough-stalked meadow grass	15 (10)	— (—)	10 (5)	20 (5)	10 (—)	14	—
Crested dogstail	— (—)	20 (10)	10 (5)	— (—)	25 (5)	16	—
Wild white clover	10 (4)	10 (4)	10 (4)	10 (4)	10 (4)	9	6
Bent (*Agrostis*)	10	20	15	10	—	10	42
Yorkshire fog	20	10	5	5	—	7	6
Fine-leaved fescue	Traces	Traces	Traces	Traces	Traces	Traces	33
Miscellaneous plants	20	20	15	20	20	17	13
Total	100	100	100	100	100	100	100
Summary:							
Sown grasses	40 } 50	40 } 50	55 } 65	55 } 65	70 } 80	57 } 66	— } 6
White clover	10	10	10	10	10	9	6
Unsown species	50	50	35	35	20	34	94

* Extracted from Stapledon and Davies (3), and represents the analysis of Deral Field in 1925 after being sown in 1918. (See text.)

A comparison of the 1925 data with those of 1933 shows a remarkable botanical transformation. In 1925 the sown species totalled only 6 per cent. of the whole, in 1933 after a second ploughing and reseeding with a mixture in which improved strains of perennial rye grass were included, the seventh year sward contained no less than 66 per cent. of sown species. This follows upon the use of indigenous strains, and has no doubt been helped by an upbuilding of soil fertility following upon a second ploughing. In addition, an increase in the phosphate efficiency of the soil is likely to have taken place (artificial phosphatic manures would have been used in 1918, and again in 1925).

Attention is now directed to the unsown species. In 1925 fine-leaved fescue and bent formed 75 per cent. of the whole pasture and the field may be said to have reverted at that time to a bent-fescue pasture, and even so it showed an improvement upon its original condition prior to 1917. In the 1933 sward of equal age, but following upon a second ploughing and reseeding with indigenous strains, the percentage of fine-leaved fescue was negligible, while bent had been reduced from 42 to 10 per cent. This evidence is striking enough and shows that while bent, fine-leaved fescue and fog are "followers of man" in the sense that they usually make an unsown appearance following upon ploughing, phosphating and reseeding, yet the use of persistent strains of grasses and clovers in the seeds mixture will largely inhibit their spread. These three grasses are propense to most of our poorer soil types, but only become aggressive in proportion as the sown species weaken and die out of the pasture.

Table VI.

Molinia *Field* (*Gwarafog*). *Botanical analyses on wet land of low fertility. Estimated percentage frequency tillers, treated and untreated swards. Seed and manure sown* 1931. *Analysed seeding year* (1931) *and in second harvest year* (6. viii. 33).

	Seeding year (1931)		Second harvest year (1933)		
Species	Plot 2, P_2O_5 only (no seed)	Plot 4, P_2O_5 + seed	Plot 2, P_2O_5 only (no seed)	Plot 3, seed only (no P_2O_5)	Plot 4, P_2O_5 + seed
Perennial rye grass*	—	—	—	—	Trace
Rough-stalked meadow grass*	—	—	—	—	1
Crested dogstail†	—	—	—	—	21
Yorkshire fog†	—	26	8	5	29
White clover†	Trace	6	Trace	2	34
Other legumes*	1	5	1	Trace	Trace
Bent (*Agrostis*)	38	22	9	8	9
Molinia *Nardus*	33	22	47	57	3
Other plants	28	19	35	28	3

† Main species in the seeds mixture (trade cleanings).
* Useful species also occurring to some extent in the seeds mixture.

(6) Molinia *Field* (*Gwarafog*) *sown* 1931.

Table VI shows the botanical composition in 1931, and again more fully in 1933, of this field which was surface cultivated in the manner already described. It was phosphated in the spring of 1931 and sown with trade cleanings containing wild white clover, dogstail and fog, together with a small proportion of perennial rye grass and wild red clover. One representative section of the field was left unseeded, while another was seeded, but not phosphated. The remainder of the field was both seeded and phosphated[1].

The original herbage consisted chiefly of *Molinia*, *Nardus* and miscellaneous plants, prominent among which were sedges (*Carex* spp.), rushes (*Juncus* spp.) and Devil's bit (*Scabiosa succisa*). The field was too rough and too wet to plough, the soil being of thin peat overlying retentive clay. The area well examples the type of problem known to us as the Cilmery "wet land" problem. Considerable areas of this type occur in western Britain and often occupy the more fertile, though heavy and retentive, soils in many districts. There is now no doubt that such areas of wet land are economically improvable.

The botanical evidence presented in Table VI shows that drastic surface cultivation followed by reseeding with an efficient mixture, together with the liberal use of phosphates, has promoted a remarkable change in the pasture make-up; a change from *Molinia* dominance and marked legume deficiency to white clover dominance with almost co-dominance of dogstail and fog.

The 1931 (year of treatment) data show that there was an immediate response, particularly marked in the high figure for fog, which evidently was able to establish itself very rapidly. Considering the 1933 data on the areas which were both phosphated and seeded it is seen that white clover, dogstail and fog contribute in the aggregate 84 per cent. of the sward, whereas their aggregate contribution on the phosphate-only and (or) seeded-only plots is not more than 8 per cent. The corresponding fall in the percentages of *Molinia*, *Nardus* and other plants of low relative value is highly significant. The proportion of bent does not seem to have been greatly affected up to date by any of the treatments. Bent is, however, known to be aggressive on these areas. If the sown species, namely, white clover, dogstail and fog, fail to persist in the coming years, it is highly probable that bent will become aggressive. This trend can be inferred from a study of the Cilmery evidence in general, and from that presented in the 1925 report (3) in particular. Surface cultivation accompanied by liberal phosphating must have improved the standard of soil fertility, and our evidence shows that the successional trend in such cases is for bent to become aggressive in proportion as the sown species die back. If this be followed by a prolonged period of soil depletion without further replenishment of plant foods, then the succession is likely to go further and the pasture to revert to one in which sheep's fescue, then *Molinia* and ultimately *Nardus* will assume dominance. From our past experiences, however, we can infer, and that with a considerable degree of confidence, that once the phase of white clover dominance has been reached and successfully maintained for a few years it will then be fairly easy to maintain grasses of relatively high fertility requirements. These will be introduced normally after a ploughing, but there is a good deal of evidence to show that they may be introduced after drastic surface cultivation, but always subsequent to the "dominant-clover phase."

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[1] A small corner was in fact left wholly untouched, but this does not enter into the present discussion as it cannot be considered representative of the field as a whole. Its composition in 1933 was as follows: *Molinia* 50 per cent.; *Nardus* 4 per cent.; total legumes 1 per cent.; other plants 45 per cent.

(7) *Warren and Ectroma Fields.*

The sward history of these two fields calls for comment. The fields lie adjacent to one another. They are on a damp retentive soil and in 1915 were described as birch-alder scrub with open patches infested either with bracken (drier knolls) or with rushes. Warren was ploughed in 1920 and sown to seeds under oats. Ectroma was gradually cleared of scrub and sown out, part in 1921 and part in 1922, each time under oats. Detailed botanical analyses were made on each in 1925. Both fields were resown in 1927 using an old pasture mixture made up approximately as follows (in lb. per acre): indigenous perennial rye grass 14; rough-stalked meadow grass 2; dogstail 2; wild white clover 3; wild red clover 4; birdsfoot trefoil 1. There was also sown a small amount (about 1 lb. per acre) of trefoil (*Medicago lupulina*). Past experience has shown us, however, that this legume is of little use on acid soils in western Britain.

Table VII.

Warren and Ectroma. Summarised botanical analyses made in 1925 *and* 1931 *on the same areas sown respectively in* (*a*) 1920–22 *and* (*b*) 1927. *Estimated percentage frequency tillers.*

	Average Warren and Ectroma	
Species	1925*	1931
Sown grasses	2	31
Sown legumes	4	11
Aggregate bent, fog and fine-leaved fescue	86	55
Other plants	8	3
Total	100	100

* Extracted Stapledon and Davies (3).

The data summarised in Table VII are again in striking confirmation of those discussed earlier in this paper. These two fields have been difficult to maintain at a high standard of botanical composition, bent being highly aggressive on them. It is clear, however, that the sown grasses from the 1927 sowing are persisting well with a corresponding decrease in the propense unsown grasses. It may be, of course, that yet another ploughing will be needed in a few more years in order to stabilise these fields at the desired rye grass, dogstail, white clover standard of excellence. Certain it is now that that object is attainable.

Photographs.

Plate XIX contains three photographs dealing with swards on the estate. Figs. 1 and 2 each represent a piece of pasture approximately 15 × 10 in. size, whereas Fig. 3 represents an appreciably larger area, as may be judged by the relative size of the clover leaves (compare Figs. 2 and 3), while every care was taken to illustrate as truly as possible the relative differences in the pastures concerned, yet having regard to the smallness of the actual area photographed it is clearly not possible that the photographs give a true picture of average botanical composition. That is one serious drawback to the use of photography in field work, namely, that the sample photographed is generally small in relation to the whole subject dealt with and therefore cannot be exactly representative of the latter.

The data embodied in this paper give for these, and other obvious reasons, a more accurate statement of the influence of treatment upon botanical composition in the pastures concerned. The photographs, however, should assist the reader to picture that which is implied throughout the written text.

GENERAL CONSIDERATIONS AND SUMMARY.

Evidence has been brought forward to show that outrun fescue-bent pastures characteristically devoid of clovers can be improved to a high stage of botanical composition at which stage perennial rye grass, white clover and certain other associate plants are dominant.

This improvement has usually been brought about in two stages. After the initial ploughing, phosphating and reseeding the trend of sward succession was always towards bent dominance with some proportion of white clover. The sown grasses (commercial strains) failed to persist. After the second ploughing at which phosphates were again applied and indigenous strains of the grasses had become available for use it has been possible to maintain, even to the ninth harvest year, swards in which the sown species still contribute well in excess of half the sward. Bent and fog are in a correspondingly subordinate position, whereas the fine-leaved fescues (which were dominant in the original pastures and at least subdominant in the once ploughed swards) are now present in negligible proportions.

A comparable tendency is to be seen with regard to a neglected wet field; there are other similar examples to be found on the Cilmery Estate. The original rough pasture was composed of *Molinia*, *Nardus*, etc., and this has in two years been transformed into one in which white clover is dominant with dogstail and fog assuming almost equal proportions to it. These three species together contribute over 80 per cent. of the sward. Traces of both perennial rye grass and rough-stalked meadow grass have appeared in this pasture, showing that the habitat is already becoming conducive to the growth of high fertility demanding grasses.

ACKNOWLEDGMENTS.

My thanks are due to Miss A. A. Poulter for help freely given in the collection of data embodied in Tables III, VI and VII, and also to Miss Rhoda Peter Jones for kindly correcting my manuscript. Grateful acknowledgment is also made to Mr Trefor Thomas, to Mr S. M. Bligh and to Prof. R. G. Stapledon for the continued and critical interest they each take in these studies.

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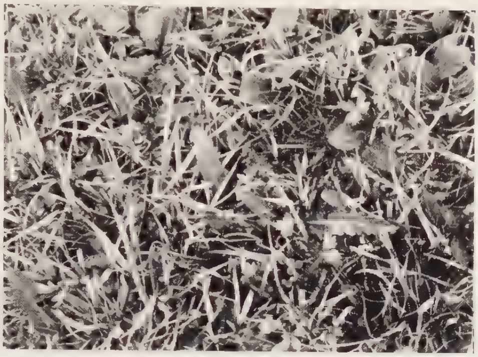

Fig. 1.

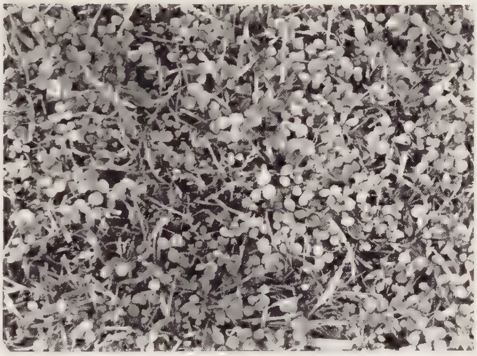

Fig. 2.

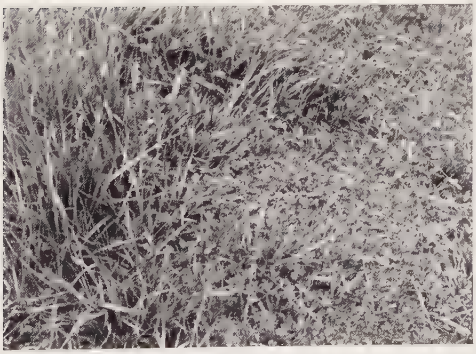

Fig. 3.

DAVIES.—Grassland Experiments in Mid-Wales; The Cilmery Park Experiments (pp. 379–397).

EXPLANATION OF PLATE XIX.

Fig. 1. Forlo Field; Cilmery. Unimproved pasture, mainly *Molinia*, Devil's bit, *Carex* and *Nardus* (these aggregate 75 per cent. of the sward). Note the trace of white clover. Close view photo taken 4. viii. 33.

Fig. 2. Forlo Field; Cilmery. Improved pasture. Surface cultivated, phosphated and sown with cleanings. About 20 yards distant from Fig. 1, on as near as possible the same soil. Mainly white clover with dogstail, rye grass, fog and bent. On this area *Molinia, Nardus* and flatweeds are all but absent. Treatment 1931: close view photo taken 4. viii. 33.

Fig. 3. *Molinia* Field; Gwarafog. Junction treated and untreated swards. Right: surface cultivated, phosphated, seed sown; white clover, dogstail and fog. Left: surface cultivated but not phosphated and no seed. *Molinia, Nardus* and *Carex*. Treatment 1931: close view photo taken 4. viii. 33.

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THE EFFECT OF RUBIDIUM SULPHATE AND PALLADIUM CHLORIDE ON THE GROWTH OF PLANTS

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(With Plate XX and 7 Text-figures.)

The complexity of the factors bearing on plant growth has long been recognised, and problems relating to the effect of environmental conditions and the importance of various elements in the nutrition of the plant have engaged the attention of investigators for many years past. More recently it has become increasingly apparent that the elements concerned in the well-being of plants are more numerous than was originally realised, and that very small quantities of certain elements may be as vitally necessary as the larger amounts of nitrogen, phosphorus, potassium and others which have long been known to be essential. The poisonous nature of compounds of copper, aluminium, zinc, etc., has been recognised and in some cases turned to account, as in the use of Bordeaux mixture for protecting potatoes against attacks of *Phytophthora*, and of copper sulphate for the destruction of charlock amongst crops. It is also well known that the presence of large proportions of certain elements as zinc in soils is inimical to plant life, though some species possess a measure of immunity and flourish in such situations (24). On the other hand, small quantities of various compounds are credited with exercising a stimulating action on growth, though much of the evidence is conflicting owing to fundamental differences in the way the experiments have been carried out.

Quite apart from stimulation it is fully accepted that small amounts of boron (11, 15) and manganese (20) are essential for the full development of many plants, and similar claims have been made for various other elements. It is quite within the bounds of possibility that some of the many obscure plant diseases or growth failures may prove to be due to the presence or absence of some element of whose importance we have at present no knowledge. Possibly, too, an element essential to one plant may not be the same as that necessary for another, though this is at present entirely in the region of hypothesis. The available information is in reality very scanty, as comparatively little work has yet been done on many of the less plentiful elements occurring in various soils. Where such an element as rubidium is known to occur in appreciable quantity, information as to its action on plant growth might be of direct importance from an agricultural standpoint, and, as a matter of fact, enquiries with regard to this element have already been received.

From another point of view these less common elements need consideration. From time to time in the development of mining and industrial concerns, stores of certain elements tend to accumulate as by-products, for which a profitable commercial outlet is sought. Or, a particular element may be found as a constituent of fertilisers (as vanadium in some basic slags), when its action on plant growth becomes a matter of considerable economic importance. It is very desirable, therefore, that our information should be increased by work on as many elements as possible, even though the immediate practical application of the results may not be apparent. For this reason, therefore, rubidium and palladium have been examined with regard to their action on various plants, rubidium being specially chosen because of its close relation to potassium.

Water culture experiments with rubidium and palladium salts were carried out at Rothamsted in 1933, cereals being started in February, followed by leguminous crops in August. Pedigree seeds of barley (Spratt Archer), wheat (Little Joss), and oats (Starr) were provided by the courtesy of the National Institute of Agricultural Botany, Cambridge, and grown at first in a modification of the usual Rothamsted solution, *p*H 5·0, with half the usual amount of calcium. Later on in the summer, when the weather was very hot, all the phosphate was supplied as potassium di-hydrogen phosphate, giving a solution of about *p*H 3·8, which allows more healthy growth in cereals under sultry greenhouse conditions. Peas (Sutton's Pioneer) were grown in the same acid solution, but with the usual full supply of calcium, while the broad beans (Sutton's Prolific Longpod) were provided with one in which the mono- and di-hydrogen phosphates were adjusted to give a *p*H 6·2. These later variations were made to provide solutions known to be favourable either to growth conditions or the plants concerned (see table on p. 400).

In order to provide some definite means whereby, if necessary, the action of one element can be compared with that of another, molar solutions were taken as a basis. As these take into consideration the

	Cereals			
	Early gm.	Late gm.	Peas gm.	Beans gm.
Potassium nitrate	1·0	1·0	1·0	1·0
Magnesium sulphate	0·5	0·5	0·5	0·5
Sodium chloride	0·5	0·5	0·5	0·5
Calcium sulphate	0·25	0·25	0·5	0·5
Potassium di-hydrogen phosphate	0·48	0·5	0·5	0·38
Potassium mono-hydrogen phosphate	0·108	—	—	0·27
Ferric chloride	0·04	0·04	0·04	0·04
Manganese sulphate	—*	0·001	0·001	0·001
Boric acid	0·001	0·001	0·001	0·001
Water (distilled)		To make up 1 litre		

* Manganese sulphate was not given at first but was added later, as the oats in particular began to show signs of deficiency.

valency of the elements, they provide a better basis of comparison than that of equivalence according to the atomic weight of the element. It was decided that in all cases a standard concentration of 1 c.c. $M/100$ per litre nutrient solution should be tested, other concentrations ranging above and below this as required. For convenience of reference this solution of $M/10^5$ is designated throughout as k.

The palladium as received was in the form of chloride, known to be a mixture of $PdCl_2$ and $PdCl_2 . 2H_2O$, so the material was dissolved and the palladium estimated, the requisite calculations then being made on the amount of the metal present. As earlier tests had shown palladium to be definitely toxic, a range of $k/32 \times 2$ to $2k$ was adopted for cereals (with $4k$ for barley in addition), and modified to $k/16 \times 4$ to $4k$ for peas and beans tested against controls without palladium.

Rubidium was used in the form of sulphate. In this case a wider range of concentrations was adopted, $k/125 \times 5$ to $125k$ for cereals, changed to $k \times 10$ to $100k$ for peas and beans, tested against controls. In all cases each individual species was examined with both elements rendering it possible to compare the response from the basis of the $M/10^5 = k$ solution.

RUBIDIUM.

Rubidium is known to occur in some soils in very small amounts (26, 31) and it has also been found in various plants, special methods being necessary for its quantitative determination in the presence of an excess of potash (21, 27, 33). As early as 1862 Grandeau (14) had determined the presence of rubidium in beetroot, tobacco, raisins and notably in coffee and tea, and concluded that the element was taken up from the soil and was probably widespread in the vegetable kingdom.

Pfeiffer (22,23) analysed beet ash in northern France, obtaining about 1·75 gm. of rubidium chloride per kilogram of ash, from which he calculated that 255 gm. of rubidium chloride were taken out per hectare with every crop of beetroot. The ratios of the rubidium chloride to sodium and potassium chloride were as 1 : 126 and 1 : 331. Lippmann (16) again verified the presence of the element in beetroot. More recently Robinson, Steinkoenig and Miller (28) determined the presence of rubidium in many plants, and found that it was not always a constant constituent of any one species, as it might be entirely absent, or present in appreciable amounts in different samples of material. They concluded that rubidium was absorbed by plants if it is present in the soil solution, but they obtained no evidence of any fertilising value.

The physiological importance of rubidium is not very clear. In spite of the close relationship between the two elements, rubidium cannot replace potassium in the metabolism of the plant. Loew (17) found that though rubidium chloride permitted normal growth of buckwheat up to flowering time, changes in the chlorophyll, accumulation of sugar and various hindrances to normal metabolism then occurred and gradually killed the plant. Rubidium nitrate caused starch accumulation, resulting in thickening and twisting of the stem, with curling and fleshiness of the leaves, and the plants died before the production of flowers. Later on Loew (18) expressed his belief that rubidium can replace potassium in the nutrition of some of the lower fungi. On the other hand, in the complete absence of potassium *Aspergillus niger* produces no spores in the presence of rubidium chloride (29,32) and the total weight of the mould is reduced, but the addition of potassium at a later stage results in spore production. Arndt (3) also found that buckwheat grown with rubidium salts instead of potassium showed definite signs of injury, such as premature dying of the roots, curling of leaves and bleaching of chlorophyll. The symptoms were allayed or removed by the addition of potassium salts, and it was inferred that the injurious action of the rubidium salts was due solely to a disturbance of metabolism, and that antagonism exists between potassium and rubidium if both are available together.

More recent work (4) with oats suggests that rubidium interferes with potash assimilation even though it may have no specific toxic effect. Alten and Gottwick (1) state that the gradual substitution of rubidium for potassium was deleterious to the growth of oats and tobacco in soil cultures, causing decreased yield and depression in various ash constituents, the harmful effect being attributed to disturbances in the equilibrium of the cell contents. The dry weight of tobacco was reduced by the presence of as little as 0·009 per cent. rubidium oxide in the soil. Attempts to replace potassium by rubidium for maize and barley grown in water cultures were unsuccessful(30), the yields falling gradually to zero as the proportion of rubidium to potassium increased.

Another aspect of the physiological action of rubidium is that of stimulation. Rubidium salts at 0·2 per cent. concentration are said to hasten the germination of barley(6), and the production of roots on cut stems of *Alternanthera spathulata* has been stimulated(2). While low concentrations of rubidium salts may improve growth(5,13,19,25,34) it is usually found that greater strengths cause damage and retard growth even if they do not kill the plants. A characteristic feature noticed by various workers in the latter cases is checking of root growth, often associated with swelling of the roots just behind the tip, recorded by Bokorny for peas, Wolkenhauer for oats and Alten and Gottwick for tobacco.

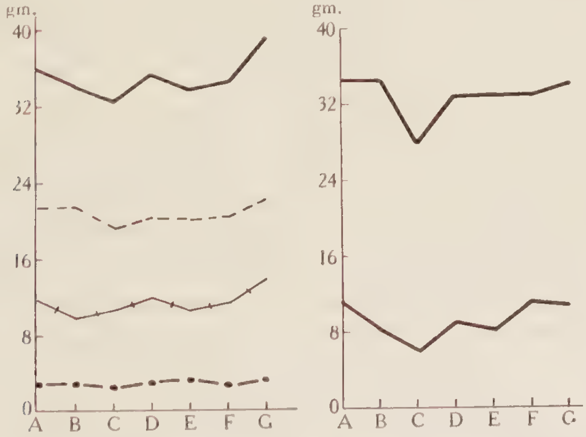
The 1933 Rothamsted experiments were designed to determine the action of a wide range of concentrations of rubidium sulphate on various plants in the presence of an adequate supply of normal nutrients including potassium. The aim was not to determine the toxicity of large amounts of rubidium, but to examine the possibility of improvement in growth.

All the cereal seeds were graded in weight, sown on February 17th in clean moist sawdust, and transferred to nutrient solutions on March 1st. After the first five weeks the solutions were changed fortnightly, with fresh supplies of the test substances, until harvest (barley July 20th, oats July 21st, wheat August 2nd). The plants were randomised in five blocks containing one replicate of each concentration, to avoid growth differences due to position which would prevent statistical estimation of the standard error. Barley made excellent growth, but with wheat the choice of the variety Little Joss proved to be unfortunate, as the plants developed a dense prostrate habit, forming large numbers of tillers of which very few eventually grew erect and produced belated ears. In the field Little Joss may be sown either in autumn or spring, but if sowing is delayed after February there is a tendency towards a similar habit. Apparently the condition of growth in water culture encouraged this abnormal development in spite of early sowing, and the response to rubidium can only be considered in relation to vegetative growth, as very little grain was produced. The oats also failed to come up to standard in their development, and ripened hardly any grain. The cause of this is not clear, but may be associated with the type of nutrient solution, the omission of manganese sulphate during the earlier weeks of growth, or with the incidence of fungal attack on the seed coats soon after the seedlings were placed in solution. The general trend of events, however, was obviously the same as in wheat and barley, and the dry weight curve is inserted for the sake of comparison.

Table I.

Effect of rubidium sulphate on the growth of barley (means of five replicates).

			Dry weight			
Treatment ($k = M/10^5$)	No. of tillers	No. of ears	Shoot gm.	Root gm.	Grain gm.	Total gm.
A. Control	16·4	14·8	21·33	2·93	11·69	35·95
B. $k/125$ Rb_2SO_4	17·0	14·8	21·40	2·93	9·77	34·10
C. $k/25$,,	14·4	13·8	19·38	2·51	10·69	32·58
D. $k/5$,,	14·4	14·0	20·38	3·09	11·90	35·37
E. k ,,	17·2	13·4	20·13	3·23	10·33	33·69
F. $5k$,,	16·6	15·6	20·43	2·66	11·23	34·32
G. $25k$,,	17·4	16·2	22·09	2·95	13·88	38·92
		S.E.	0·98	0·22	1·019	1·94



Text-fig. 1. Dry weights of cereals grown with rubidium sulphate. Left, barley; right, wheat (upper), oats (lower). —— total; – – – shoot; ┼┼ grain; ·–·–·– root. (For concentrations A–G see Table I.)

With all these cereals no variation was observed during growth at any stage of development which could be attributed to any concentration of rubidium sulphate. In barley all root and shoot growth was normal and similar to that of the control plants, ear emergence occurred simultaneously throughout the series with no significant difference in the number of ears, and the ripened grain was all fit to harvest at the same date (Plate XX, fig. 1). With wheat and oats, also, the plants receiving rubidium sulphate developed in just the same way as the controls. These visual observations were fully borne out by the dry weights, the variation between individual plants with the same treatment or in the same randomised blocks being as great as that caused by treatment with different concentrations of rubidium sulphate, the standard errors of the results of treatment being insignificant (Table I and Text-fig. 1).

In the above experiments week old seedlings were used which had not been treated with rubidium during germination. In case this immune period might have affected the after results, barley seeds were soaked for six hours on November 30th in rubidium sulphate solutions of the same graded strengths as in the main experiment. These seeds were then germinated in beakers on perforated slabs of paraffin wax floating on 100 c.c. of similar rubidium sulphate solutions in two series, one with distilled water and the other with complete nutrient solution. Satisfactory growth was made and after six weeks there was no obvious difference in the growth of the seedlings due to increasing strengths of rubidium sulphate, thus corroborating the previous results. The method was not satisfactory with wheat, peas and beans, owing to the rapid growth of moulds on the germinating seeds.

Water culture experiments carried out in autumn with peas gave similar results to those with cereals, no significant differences due to the rubidium sulphate being observed (Table II and Text-fig. 2).

With broad beans there was an increased dry weight with the two lower concentrations which approached significance, especially in the comparison between the controls and those receiving $10k$ rubidium sulphate. As beans are so extremely variable in growth, little weight can be placed on this one instance of suggested improvement.

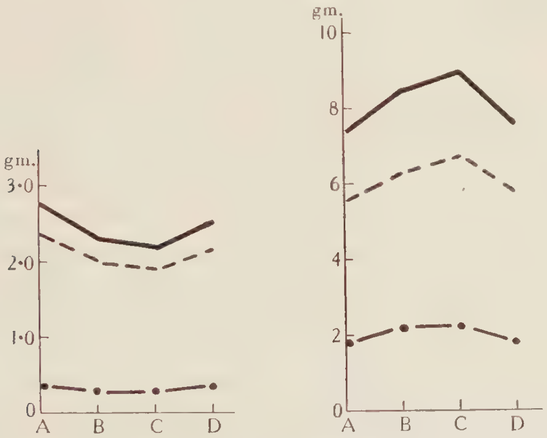
The general indication from these experiments is that in the presence of adequate supplies of potash, rubidium sulphate in concentrations of $125k = (125\ M/10^5)$ and less has no appreciable influence on growth. The maximum amount of rubidium sulphate used represented 0·01668 per cent. by weight, other workers having reported beneficial effects with much heavier doses up to 0·2 per cent. (18), and, using the chloride, with as little as 10 mg. to 50 kg. soil, equivalent to 0·00002 per cent. (23).

It would seem to be fully established that rubidium cannot replace potassium in the plant economy, but in view of the great discrepancies in the results of various workers it has not yet been definitely shown that, *in the presence of adequate supplies of potash*, large doses of rubidium salts are definitely toxic, or that very small quantities exercise a stimulating action on growth.

Table II.

Effect of rubidium sulphate on the dry weight of peas and broad beans (means of five replicates).

Treatment ($k = M/10^5$)	Peas Shoot gm.	Peas Root gm.	Peas Total gm.	Broad beans Shoot gm.	Broad beans Root gm.	Broad beans Total gm.
A. Control	2·37	0·38	2·75	5·57	1·79	7·36
B. k	2·01	0·28	2·29	6·24	2·22	8·46
C. $10k$	1·90	0·27	2·17	6·72	2·24	8·96
D. $100k$	2·15	0·35	2·50	5·75	1·80	7·55
S.E.	0·33	0·07	0·38	0·41	0·19	0·55



Text-fig. 2. Dry weights of peas (left) and broad beans (right) grown with rubidium sulphate. —— total; – – – shoot; ·–·–·– root. (For concentrations A–D see Table II.)

PALLADIUM.

Very little work has been done with palladium in connection with plant growth, as it is not one of the rarer elements which is widespread in soils and its presence in plants does not seem to have been recorded. Coupin (12) found that palladium chloride was very strongly toxic to wheat in the early stages of germination, and its toxicity to broad beans was found some years ago in Rothamsted experiments, in an attempt to replace boron by palladium in culture solutions. Its behaviour in these preliminary tests suggested the recent trials with a range of concentrations and various species of plants, as indicated in the introduction.

Barley. The series in general was very strong and healthy, and the action of the increasing concentrations of palladium showed such a marked gradation that it is advisable to set out in some detail the observations on the developmental history of the plants (Plate XX, fig. 2).

Throughout growth concentrations of $k/16$ and less had no apparent influence on growth, either adverse or beneficial, and this was corroborated by the yield figures. All higher concentrations checked development from the first, the initial degree of checking becoming more marked with increasing concentration. With $k/8$ root growth was hindered and tillering was delayed, but after about six weeks growth proceeded more rapidly and after ten weeks the plants had caught up to the controls. No further advance was made beyond the controls, and yields were parallel in both cases.

With twice this strength of palladium $k/4$ the initial check was much more severe, and recovery considerably later. At an early stage the deficient functioning of the roots was shown by a reddening of the shoots, a very usual indication of starvation which can occur in the midst of plenty if absorption is hindered. Eventually improvement occurred, though the plants throughout appeared weaker than the controls.

From this concentration upwards the toxic effect of palladium was much more definite, and distinct morphological differences were manifested. The initial check was almost complete, the red colour of the stem was very marked, and when growth did occur the development was abnormal. With $k/2$ the roots grew very slowly and were short, thick and stubby in nature, evincing an apparent reluctance to enter the solution. Here again improvement set in after about six weeks. Longer and thinner roots grew out, tillering began, and the plants slowly assumed a more normal and characteristic appearance, but the progress of ripening was delayed and the ears did not change colour so early as those of the controls.

With k the primary check was more severe and lasted longer before progress began. The tips of the stubby roots showed curious thickenings, recalling those mentioned by Wolkenhauer with rubidium (21). These were quite firm in texture, unlike the flabby thickenings which sometimes appear when roots come in contact with toxic solutions. Tillering was considerably delayed, as neither shoots nor roots made any definite headway for more than six weeks after the experiment was set up, and the ultimate yield was much reduced, while the grain was still green at the time of harvest.

With $2k$ the same thickenings occurred at the root tips, but at first it appeared that no recovery would be made. However, between the sixth and tenth week amazing progress occurred, a good number of tillers were produced and fairly good roots put forth, though the latter were thicker than normal, after which growth proceeded steadily and a comparatively good yield resulted. The effect of $4k$ concentration was in some respects the most surprising of all. The initial check was so severe that the plants seemed almost dead; no root movement occurred at all, and the shoot remained practically undeveloped. So bad seemed the condition that the discarding of the plants was considered. This state of utter stagnation continued for over six weeks, after which signs of renewed vitality became manifest. A month later some degree of root development had occurred in several plants, coupled with the production of a few tillers. These plants remained very small, but most of them developed one or more earing shoots, though grain production was very feeble and the ears were quite green at harvest.

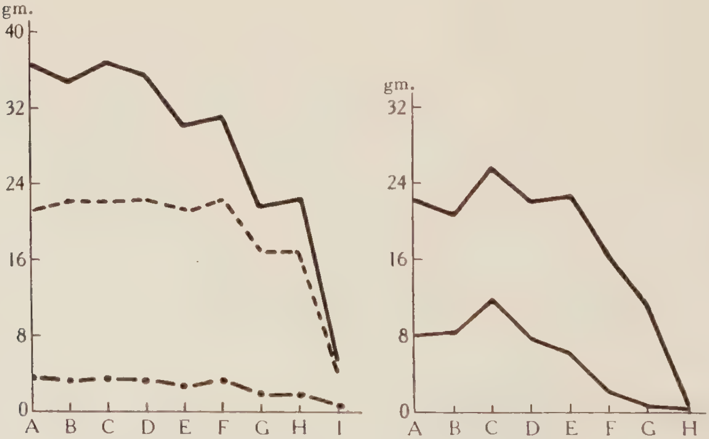
This recovery in strong concentrations of inorganic poisons was unexpected. When working with other elements, as copper, zinc, arsenic, etc., it had usually been found that if the toxic compound is sufficiently concentrated to cause severe check to growth, no ultimate recovery occurs if this primary check is at all prolonged (7, 8). Recovery with gold and mercury was, however, observed in broad beans, but the period of stagnation was much shorter (11). On the other hand, with organic compounds as hydrocyanic acid (9) and phenols (10) some measure of recovery frequently occurs after a dormant period of a month or more, particularly when the nutrient solutions are not changed too often, provided the poison is not so strong as to kill the plant immediately. This had been attributed to a decomposition of the organic compounds into less harmful substances, but in the case of inorganic salts a similar disorganisation of the toxic substance does not occur, as the poisonous property is associated with the element itself. A possible explanation of the behaviour of barley in strong palladium chloride solution may lie in the change in climatic conditions during growth. The summer of 1933 was unusually hot and sunny, and a definite and rather sudden rise in the maximum and mean temperatures occurred towards the end of April, when the maximum was frequently 80° F. instead of 70° F. as was usual in the earlier part of the experiment. This increase in temperature coincided rather closely with the general impaired growth of the barley which was adversely affected by palladium, and may have provided the impetus which enabled even the most badly checked plant in $4k$ to produce at least a modicum of normally developed roots and shoots.

The plants were harvested and dried when the controls had ripened their grain. The three weakest concentrations had no effect on the dry weight, but with stronger solutions a depression set in, becoming more marked with increasing palladium until with 4*k* the total dry weight was only 5·3 gm., representing but 14·4 per cent. of that of the control (Table III and Text-fig. 3).

Table III.

Effect of palladium chloride on barley (means of five replicates).

	Dry weight					
Concentration ($k=M/10^5$)	Shoot gm.	Root gm.	Grain gm.	Total gm.	No. of tillers	No. of ears
A. Control	21·23	3·56	11·92	36·71	17	15
B. *k*/32	22·07	3·27	9·47	34·81	17	16
C. *k*/16	22·01	3·45	11·35	36·81	21	16
D. *k*/8	22·16	3·22	10·05	35·43	15	15
E. *k*/4	21·23	2·59	6·06	29·88	17	15
F. *k*/2	22·15	2·93	5·97	31·05	25	15
G. *k*	17·13	1·83	2·42	21·38	22	14
H. 2*k*	16·68	1·85	3·71	22·24	17	13
I. 4*k*	4·41	0·66	0·23	5·30	6	3
S.E. excluding 4*k*	1·67	0·30	1·26	2·96		

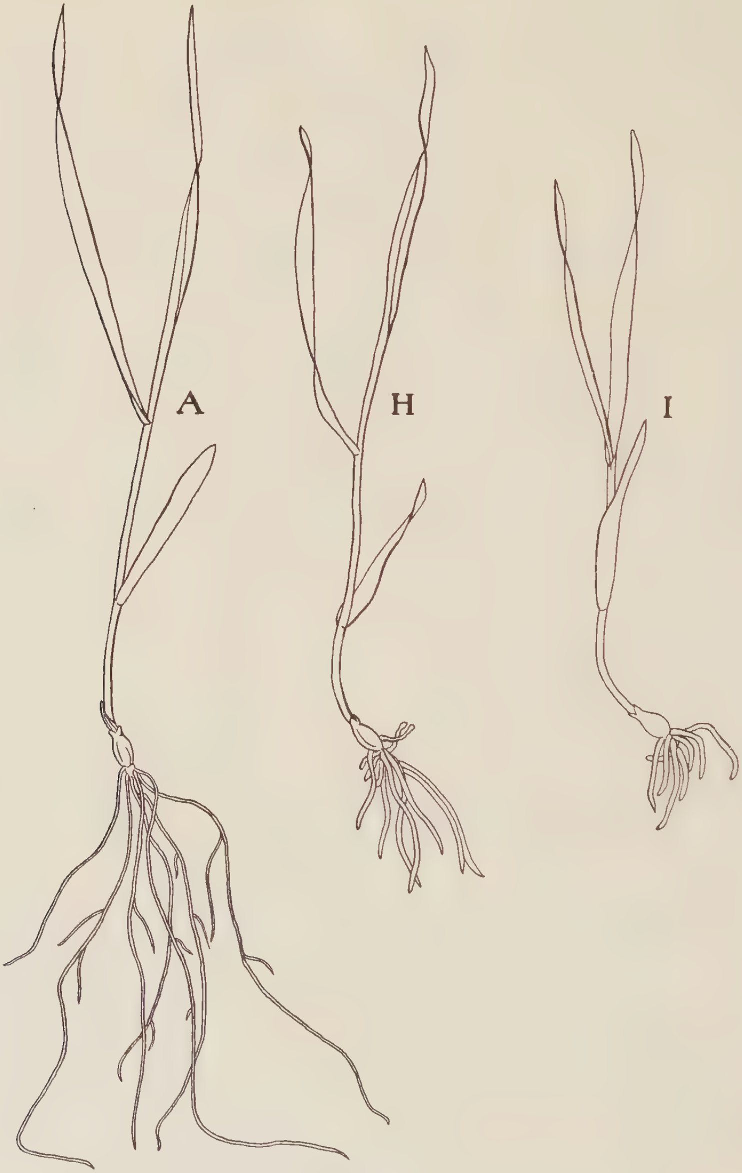


Text-fig. 3. Dry weights of cereals grown with palladium chloride. Left, barley; right, wheat (upper), oats (lower). —— total; – – – shoot; · – · – · – root. (For concentrations A–I see Table III.)

The response of the different plant organs, however, was not the same, some being more influenced than others. The roots showed certain variable fluctuations in the weaker strengths, but no significant drop was indicated until *k* was reached, and though the shoots appeared to respond at the same point statistical examination of the individual results show that the differences were not significant, except for the sudden drop with $4k$. Actually, however, a definite adverse action was evident in spite of the non-significance of the dry-weight figures from one concentration to another. The indicated drop in the k and $2k$ shoots was due to certain plants which failed to recover from the initial check so completely as their fellows, suggesting that at about these concentrations the degree of shoot recovery depends upon the inherent vigour of the individual plants. It is tempting, also, to attribute the heavy tillering with $k/2$ and k to the action of the poison, but in view of a corresponding increase with $k/16$ it is unsafe to make this assumption. The total number of ears was not significantly affected except with the strongest concentration. The grain showed much more definite reaction to the palladium, significant decrease in dry weight setting in with $k/4$, after which the decreasing weight with increasing concentration was associated with a corresponding delay in ripening, many of the ears being still quite green when the controls were dead ripe. Germination tests on barley were carried out as with rubidium. During the first week the amount of growth decreased steadily as the palladium solutions became stronger, from the lowest concentrations upwards. The less checked plants gradually caught up the controls, but with the strongest concentration this recovery did not occur during the six weeks of the experiment, and the roots with $2k$ and $4k$ made very little growth (Text-fig. 4). The general trend of events was similar in both series, but in the absence of nutrient salts the weaker concentrations of palladium chloride were somewhat more toxic and recovery was not so complete.

Wheat. In the early stages the weakest concentration which checked growth was $k/8$ as in barley, the set-back becoming more marked with increasing palladium until with k and $2k$ there was practically no development. With the weaker concentrations the degree of recovery with time was very parallel to that in barley, but with greater strengths wheat did not make such good progress, and with the strongest concentration very little growth occurred throughout (Text-fig. 3), whereas under similar conditions barley plants produced about half as much dry matter as the controls.

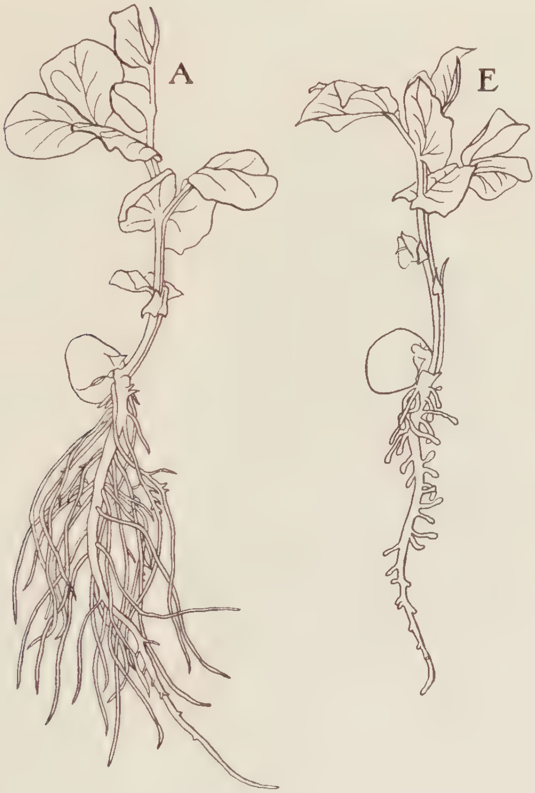
Reference has already been made to the abnormal character of the growth of wheat. Tillering was very heavy, but when the experiment was concluded the numerous early tillers had died down leaving the few green shoots which carried unripe ears. At this stage no check was evident with $k/4$, but as the palladium increased to $2k$ the harmful action



Text-fig. 4. Effect of palladium chloride on the germination and early growth of barley in nutrient solutions. (For concentrations A, H, I see Table III.)

became very strongly marked in total dry weight, number of tillers and ear production. The thickening and stubbiness of roots, characteristic of poisoning, was manifest in all the checked plants.

Oats. Oats gave signs of being more sensitive than either wheat or barley to the harmful action of palladium, as growth was almost entirely checked at concentrations which allowed good growth in the other cereals. The development, however, was very poor, and it is possible that this prevented the recovery from toxic effects which might have occurred in stronger plants.

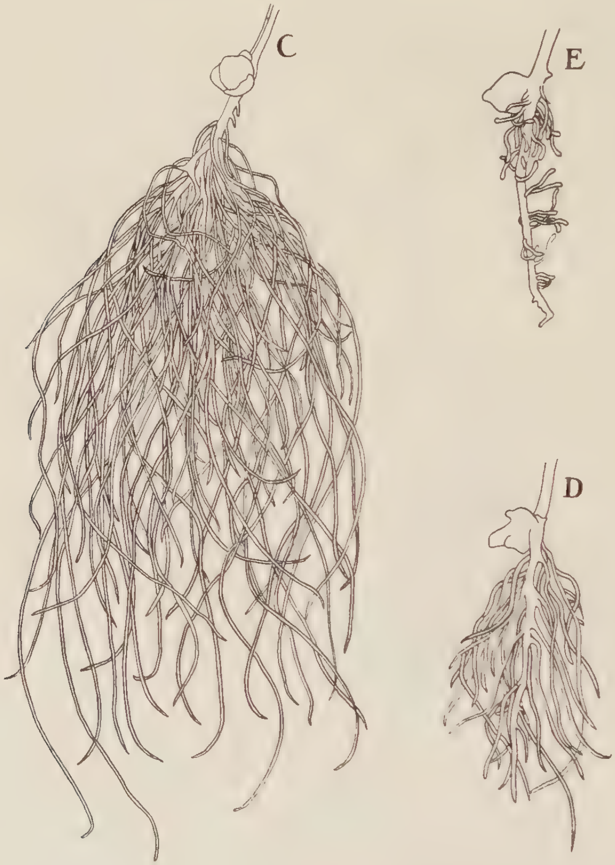


Text-fig. 5. Effect of palladium chloride on peas, after eight days in solution. A, control; E, with strong solution. (For concentrations see Table IV.)

The initial checking was again evident with $k/8$, progressive injury occurring with increasing concentration (Plate XX, fig. 3). Abundant root-hairs were a noticeable feature of the most strongly poisoned plants.

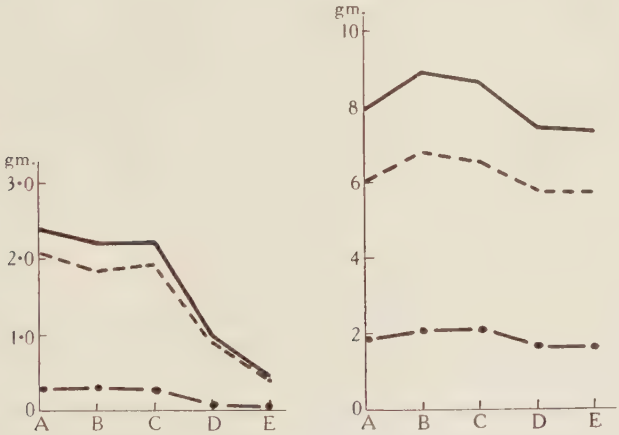
As time went on stubby roots were manifest from $k/4$ upwards, being more marked at lower concentrations than in wheat or barley.

By harvest time all the plants had developed a certain number of panicles, which had changed colour and ripened off, but hardly a fertile grain was produced, even the controls having none.



Text-fig. 6. Effect of palladium chloride on peas, after seven weeks in solution. C showing complete, and D partial, recovery from initial check. (For concentrations see Table IV.)

Peas. Peas were grown in late summer and showed the great toxicity of the higher concentrations of palladium. From the first the heaviest dose ($4k$) drastically checked the roots which became yellow and produced stubby knotted laterals (Text-figs. 5 E and 6 E), the shoots being correspondingly small. In this case there was no recovery as time went on. With one quarter the strength (k) a certain amount of lateral root growth ultimately occurred (Text-fig. 6 D), but the plants were seriously damaged, the shoots being small and the roots yellow. With the weaker concentrations, however, the initial check was completely overcome (Text-fig. 6 C) and the final development was as good as that of the control plants receiving no palladium. When harvested after two months' growth the roots in the two strongest concentrations were still stunted and very dark or black, the shoots being small and dying. Even the most badly poisoned plants, however, had flowered and were forming pods. The drop in dry weight was sharper in the shoot than in the roots (Table IV, Text-fig. 7), and the total production with $4k$ palladium was only about 20 per cent. of that of the controls.



Text-fig. 7. Dry weights of peas (left) and broad beans (right) grown with palladium chloride. —— total; – – – shoot; ·–·–·– root. (For concentrations A–E see Table IV.)

Table IV.

Effect of palladium chloride on the dry weight of peas and broad beans (means of five replicates).

	Peas			Broad beans		
Treatment ($k = M/10^5$)	Shoot gm.	Root gm.	Total gm.	Shoot gm.	Root gm.	Total gm.
A. Control	2·08	0·31	2·39	6·04	1·86	7·90
B. $k/16$	1·85	0·37	2·22	6·80	2·09	8·89
C. $k/4$	1·93	0·28	2·21	6·56	2·11	8·67
D. k	0·92	0·07	0·99	5·77	1·68	7·45
E. $4k$	0·39	0·04	0·43	5·72	1·61	7·33
S.E.	0·21	0·045	0·27	0·53	0·16	0·67

Broad beans. This was the only species in which the toxic action of the stronger concentration of palladium was not very noticeable. In the early stages the plumules and roots were somewhat stunted with heavy doses of palladium. The shoots made a complete recovery and developed normally. Some thickening and shortening of the roots persisted throughout growth, but was not very marked. The dry weights showed no significant differences due to treatment, the variation between individuals being greater than that due to palladium (Table IV, Text-fig. 7).

Summing up the general results, as far as the three cereals are concerned the action of palladium appears to be very similar, with certain differences in the range at which the toxic action is manifest. In all cases an early check with comparatively low concentrations occurs, followed by a gradual recovery, which does not necessarily affect all the plant organs in the same degree, the roots and grain, in barley at least, being more influenced than the shoot. The poisoned roots become thick and stubby, entering the solution with reluctance, but later in life considerable recovery towards normal occurs, such recovery being possible in barley in the presence of higher concentrations of palladium than with either wheat or oats.

SUMMARY.

1. Comparisons have been made between the action of rubidium sulphate and palladium chloride on the growth of barley, wheat, oats peas and beans in complete nutrient solutions.

2. Over a wide range of concentrations *rubidium sulphate* was not found to exercise either a beneficial or a harmful action on the growth of any of the species tested. The germination of the seeds was likewise not affected.

3. No benefit was derived from *palladium chloride*, but at a comparatively low concentration a harmful action occurred which became more intense with increasing concentration. Stunting of the main root and laterals was a characteristic feature of this toxicity. With the lower concentrations the check was temporary, and the roots eventually made normal growth, as good as that in the control plants. With increasing amounts of palladium chloride the poisoning effect became more persistent, until a concentration was reached which did not allow of any root or shoot recovery.

4. The tolerance of palladium varies with the species, as was indicated by the measure of recovery. Barley appeared to be the least, and

oats the most sensitive of the three cereals tested. Peas responded at much the same concentrations as barley, but broad beans made so complete a recovery from the initial checking that the dry weights were ultimately not reduced even by the strongest concentration tested, the plants being indistinguishable from the controls.

5. The effect of palladium poisoning was similar whether the seeds were germinated in the presence of palladium or whether the seedlings were not introduced to it until they were about a week old.

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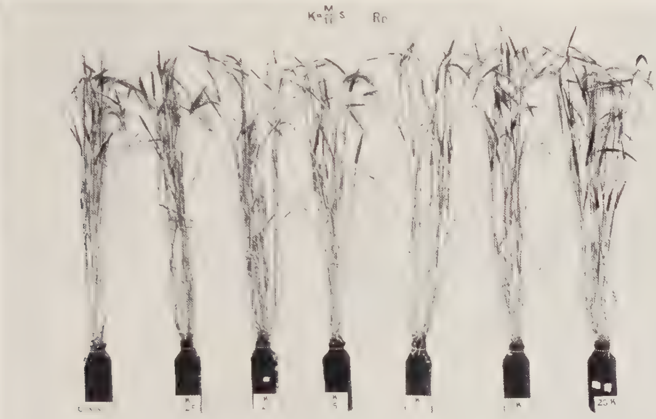

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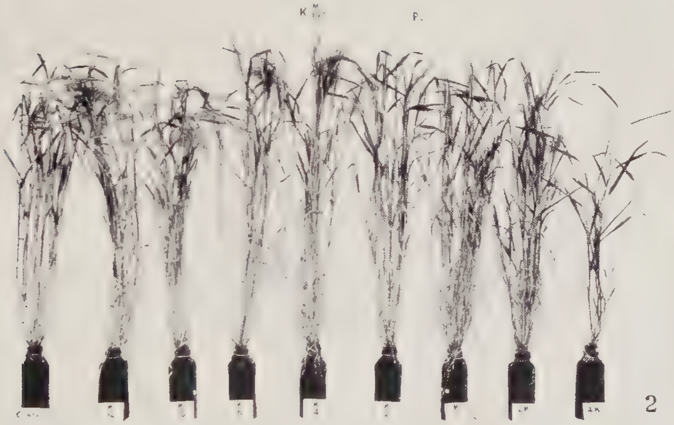

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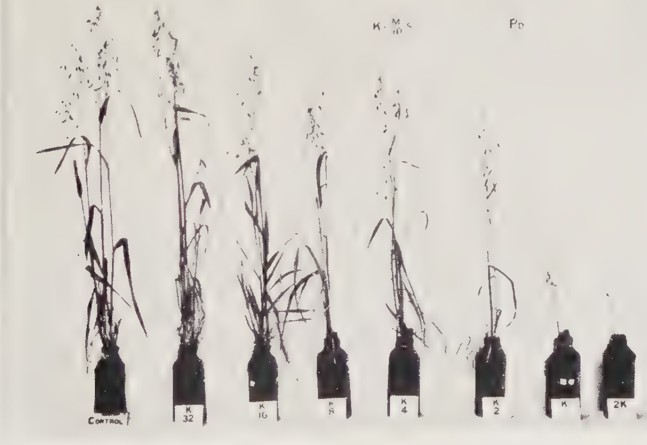
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BRENCHLEY.—The Effect of Rubidium Sulphate and Palladium Chloride on the Growth of Plants (pp. 398–417).

EXPLANATION OF PLATE XX.

Fig. 1. Effect of increasing concentrations (left to right) of rubidium sulphate on the growth of barley. (See Table I.)

Fig. 2. Effect of increasing concentrations (left to right) of palladium chloride on the growth of barley. (See Table III.)

Fig. 3. Effect of increasing concentrations (left to right) of palladium chloride on the growth of oats. (Concentrations as for barley, Table III.)

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THE SEEDLING CHARACTERS OF SOME CULTIVATED *BRASSICAE*

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(With Plates XXI and XXII.)

Introduction.

In 1931 Gorman and Lafferty (1) published a paper entitled "On a method of distinguishing the seedlings of swedish turnip (*Brassica Napus* L. var. *Napo-brassica* (L.) Reichb.) from those of rape (*Brassica Napus* L. var. *biennis* (Schubl. et Mart.) Reichb.)," in which they concluded that seedlings of the swede turnip and broad-leaved rape were different in morphological characters, the differences being such that seedlings of these two kinds of *Brassica* could be recognised with certainty.

Both plants are varieties of *Brassica Napus* L.; the swede develops a bulbous "root" which is of great value for winter feeding of cattle and sheep, while the rape plant has no bulbous "root" but produces an extensive system of leafy shoots on which sheep are folded. The seeds of both plants are very similar and cannot be distinguished with certainty, hence great care is necessary on the part of the seedsman and farmer to avoid mixture or accidental substitution of one for the other.

Gorman and Lafferty's diagnosis of the swede and rape seedlings depends upon the form of the first foliage leaf and the relative length of the first internode of the plants.

Experimental.

With a view to testing these results and determining how far the conclusions put forward could be applied to other rapes and bulb-forming Brassicas, an extensive series of observations was carried out on seedlings of the following:

(1) White-fleshed swede (*Brassica Napo-brassica* DC.).
(2) Yellow-fleshed swede (*B. Rutabaga* L.).
(3) Broad-leaved winter swede rape (*B. Napus* L. forma *oleifera*).
(4) Summer swede rape (*B. Napus* L. forma *annua*).
(5) White-fleshed turnip (*B. Rapa* L.).

(6) Yellow-fleshed turnip (*B. Rapa* L.), sometimes erroneously called hybrid turnip.

(7) Winter turnip rape (*B. campestris* L. forma *oleifera*).

(8) Summer turnip rape (*B. campestris* L. forma *annua*).

Publications (2,3,4,5,6) dealing with the descriptions and nomenclature of these and other allied Brassicas were examined and it was decided to adopt Percival's classification (7), which is considered to be more in accordance with the structure and characteristics of the plants than others which have been formulated.

Rows of seeds of each of the above species of *Brassica* were sown, (1) in a heated greenhouse, (2) in an unheated greenhouse, and (3) in the open in mid-January, and at approximately monthly intervals thereafter throughout the year. Seeds sown in the greenhouse were sown in loamy soil contained in shallow boxes. The seedlings from seeds sown in the open during January, February and March, all appeared at the same time, the temperature out of doors being apparently too low for germination during the first two months of the year. In order to establish certainty in regard to the species under investigation, and their bulb-forming or leafy-branching habit, the plants were grown on to maturity. Batches of seedlings of each species were also grown in a dark cupboard to ascertain whether there were any differences in cotyledon colour when the formation of chlorophyll was prevented.

DESCRIPTION OF THE SEEDLINGS.

In the seedlings under observation the expanded cotyledons were found to be of two main types namely, the "swede type" found in swede turnips and swede rapes, and the "turnip type" found in ordinary turnips and turnip rapes. The swede type of cotyledon is broadly oblong, notched, and each half resembles a large segment of a circle. The turnip type of cotyledon is also broadly oblong, but is much longer in proportion to its breadth than is the swede type, and the whole cotyledon is heart-shaped.

White-fleshed swede (Brassica Napo-brassica *DC*).
(Plate XXI, fig. 1.)

Cotyledons. The cotyledons are of the "swede type," rather dark green in colour and glabrous. The mid-rib and one or two pairs of lateral veins are clearly marked on the lower surface. There is usually a certain amount of violet pigment in the epidermis of the cotyledons, but this varies in plants raised from seed obtained from different sources. In some cases there is only a small amount of pigment in the notch of the cotyledon and in the veins; in others, the pigment extends right round the margin of the cotyledons, and the veins are a deep violet colour.

The petioles are smooth, slightly grooved on the upper side and convex below, green, with a variable amount of violet pigment.

In seedlings grown in the dark the cotyledons are pale yellow.

Hypocotyl. The hypocotyl is glabrous, violet above ground, shading off to white below the surface of the soil.

First leaf. The lamina of the first leaf is glaucous, and of variable shape. Common forms of lamina are:

(1) Nearly circular (*circa* 30 mm. long by 27 mm. wide) with a very broad but not markedly projecting terminal tooth, and 4–6 pairs of lateral teeth of irregular size with rounded tips, the incisions becoming deeper towards the base of the lamina.

(2) Much longer than broad (*circa* 30 mm. long by 20 mm. broad), widest at about one-third of the distance from the apex, with a more projecting terminal tooth, more pointed lateral teeth, and deeper incisions in the lamina. There are a few hairs scattered irregularly on the upper surface and along the margin of the lamina; on the lower surface hairs are found only on the veins.

The petiole is of variable length, flat on the upper surface, and convex below, broadening towards the base. In some plants it is pale green without a trace of pigment, in others it is a deep violet colour. Sometimes the petiole is quite glabrous, or it may have a few scattered hairs, usually on the back.

First internode. There is no visible first internode in about 60 per cent. of the plants, while in others the internode is 2–3 mm. long, glabrous or with one or two hairs, and of the same colour as the hypocotyl. Seeds sown in an unheated greenhouse during January and February produced plants none of which showed an elongated first internode, but when sown in a heated greenhouse during the same period, they behaved similarly to those sown out of doors in the warmer months.

Yellow-fleshed swede (B. Rutabaga *L.*).
(Plate XXI, fig. 2.)

Cotyledons. In seedlings grown out of doors and in the greenhouse, the cotyledons are similar to those of the white-fleshed swede. In seedlings grown in the dark the cotyledons are of a deep rich yellow tint.

Hypocotyl. The hypocotyl is glabrous, and of a greenish violet colour shading off to yellowish white below the surface of the soil.

First leaf. The lamina is glaucous and variable in form, the predominant shape is somewhat circular (*circa* 26 mm. long, by 24 mm. broad, widest across the middle). The leaf usually has a small terminal tooth with a large number (up to eleven) of pairs of small pointed lateral teeth. A rarer form of leaf is much longer than broad (*circa* 32 mm. long by 22 mm. broad), widest about one-third of the distance below the apex, with a large terminal tooth, and four or five lateral teeth; many are intermediate between this and the predominant form.

The leaves show a variable degree of hairiness, the hairs being scattered irregularly on the upper surface, around the margin of the lamina, and on the veins of the lower surface.

In some plants the petiole is a light green colour, in others a trace of violet pigment is present; a few hairs may be seen all round the petiole or only on the lower convex surface.

First internode. In about 60 per cent. of the seedlings raised from seed sown out of doors in May, the first internode is not visible; in others it is of variable length up to 3–4 mm., glabrous or with a few hairs, and of the same colour as the hypocotyl. Seedlings raised in an unheated greenhouse during January, February and March, show no elongation of the first internode, but when grown in the greenhouse during the warmer months, a large percentage of the plants produce first internodes up to 5 or 6 mm. in length.

Winter swede rape (B. Napus *L. forma* oleifera).

(Plate XXI, fig. 3.)

Cotyledons. In seedlings grown out of doors, in the greenhouse and in the dark, the cotyledons are similar to those of the white-fleshed swede.

Hypocotyl. The hypocotyl is glabrous, light violet in colour with a few pale-green streaks shading off to white below the surface of the soil.

First leaf. The commonest type of lamina is much longer than broad (*circa* 35 mm. long by 25 mm. broad), widest about one-third of the distance from the apex, and gradually narrowing towards the base. The terminal tooth is large, broad and projecting, and there are 3–5 pairs of somewhat sharply pointed lateral teeth. Some first leaves are widest across the middle (*circa* 32 mm. long by 28 mm. broad) with a broad but projecting terminal tooth; others are nearly oblong. In all samples the first leaves of some of the seedlings are misshapen and lop-sided.

The veins are clearly marked, pale green in colour, rarely showing any trace of violet pigment. Hairs are present on the upper surface, round the margin, and on the lower surface of the lamina.

The petiole is of a light green colour, with a trace of violet pigment in a few cases; in many plants it is quite glabrous, in others it bears a few scattered hairs.

First internode. This is not visible in about 30 per cent. of the plants; in the remainder, it is usually 3 mm. or more in length, green and glabrous. Seedlings raised in an unheated greenhouse during January, February and March showed no elongation of the first internode, but in those raised in the greenhouse in the summer months all had elongated first internodes.

Summer swede rape (B. Napus *L. forma* annua).
(Plate XXI, fig. 4.)

The seedlings are exactly similar to those of the winter swede rape.

White-fleshed common turnip (B. Rapa *L.*).
(Plate XXII, fig. 5.)

Cotyledons. The cotyledons are of the "turnip type," glabrous and of a lighter shade of green than those of swede and swede rape. The mid-rib and two irregularly placed veins on either side of it are clearly visible on the lower surface. There is usually no violet pigment in the cotyledons.

The petioles are light green, usually without any violet tinge, glabrous, and grooved on the upper surface.

In seedlings grown in the dark the cotyledons are of a pale yellow colour.

Hypocotyl. The hypocotyl is light green above the soil, white below: in some plants there is a small amount of violet pigment in the hypocotyl.

First leaf. The lamina of the first leaf is extremely variable in form. A few of the commoner types are:

(1) Widest across the middle (*circa* 32 mm. long by 20 mm. broad). The terminal tooth is not larger than the lateral teeth, of which there are five or six pairs, blunt in the upper half of the leaf, more acute in the lower half.

(2) Widest at about one-third of the distance from the apex (*circa* 40 mm. long by 24 mm. broad) with a large terminal tooth, and four or five pairs of lateral teeth; incisions deeper towards the base of the leaf.

(3) Long and narrow (*circa* 35 mm. long by 19 mm. broad), widest across the middle with a short and narrow terminal tooth, and six or seven pairs of very acute lateral teeth; incisions of the lamina somewhat deep.

The lamina is grass green, and the veins well marked. There are stiff white hairs on the upper surface, round the margin of the lamina, and on the veins on the lower surface.

The petiole is pale green in colour, sometimes with a trace of violet pigment; it is flat above, convex below, with a slight wing running nearly to the base. The upper surface is usually glabrous, but a few scattered hairs are seen on the sides and convex lower surface.

First internode. There is no visible first internode in plants grown in the open, nor in those grown in the greenhouse in January, February and March; in plants grown in the greenhouse later in the year (April and May) some developed elongated first internodes up to 3 mm. long, of the same colour as the hypocotyl, and sometimes bearing a few hairs.

Yellow-fleshed turnip (B. Rapa *L.*).

Cotyledons. In seedlings grown out of doors, and in the greenhouse, the cotyledons are similar to those of the white-fleshed turnip: grown in the dark the cotyledons are a deep yellow colour.

Hypocotyl. The hypocotyl is greenish violet, shading off to yellowish white below the soil level.

First leaf. The lamina is very variable in form, some of the commoner types are:

(1) Widest across the middle (*circa* 38 mm. long by 24 mm. broad), with a small terminal tooth and eight pairs of small lateral teeth.

(2) About 35 mm. long by 20 mm. broad, with a small terminal tooth, and eight pairs of lateral teeth almost equal in size to the terminal tooth.

(3) About 33 mm. long by 24 mm. broad, with an extremely small terminal tooth, and eight pairs of lateral teeth; the lamina is deeply cut at the base.

(4) Lyrate, with a large irregularly toothed terminal lobe and two lateral lobes.

The lamina is grass green, and the veins are well marked. There are stiff white hairs on the upper surface of the lamina, round the margin, and on the veins of the lower surface.

The petiole is usually pale green, but sometimes there is a considerable amount of violet pigment present; it is flat above and convex below, with a narrow wing on each side reaching nearly to the base. In some plants the petiole is almost glabrous, in others numerous stiff white hairs are present on the sides and lower convex surface.

First internode. The first internode is not visible in plants grown in the open, nor in those grown in the greenhouse in January, February and March. Of those sown in the greenhouse in April and May, some plants developed elongated first internodes up to 3–4 mm. long, of the same colour as the hypocotyl and sometimes bearing a few stiff hairs.

Winter turnip rape (B. campestris *L. forma* oleifera).
(Plate XXII, fig. 6.)

Cotyledons. The cotyledons are like those of the white-fleshed turnip, but of a much darker shade of green.

In seedlings grown in the dark the cotyledons are a deep yellow colour like those of the yellow-fleshed turnip.

Hypocotyl. The hypocotyl is greenish violet above ground, shading off to white below the surface of the soil.

First leaf. The lamina is very variable in form; common shapes are:

(1) Ovate (*circa* 31 mm. long by 21 mm. broad), widest across the middle, with a broad terminal tooth, and four pairs of blunt lateral teeth.

(2) Ovate (*circa* 34 mm. long by 21 mm. broad), widest across the middle, with a small terminal tooth, and four pairs of blunt lateral teeth about the same size.

(3) Broadly ovate (*circa* 25 mm. long by 20 mm. broad), with a small terminal tooth and five pairs of blunt lateral teeth.

(4) Ovate (*circa* 23 mm. long by 16 mm. broad), with a large terminal tooth, and four pairs of small, sharply pointed lateral teeth.

The first leaves are of a darker shade of green than those of the turnip (*B. Rapa* L.) but not glaucous.

The veins are prominent and occasionally contain traces of violet pigment. There are numerous stiff white hairs on the upper surface and around the margin of the lamina: on the under-surface hairs only appear on the veins.

The petiole is pale green or violet green, with a variable number of stiff white hairs on the convex lower surface and sides.

First internode. In 90 per cent. of plants grown out of doors the first internode is not visible, but in a few plants it is slightly elongated (1–2 mm. long), the same colour as the hypocotyl, and clothed with stiff white hairs.

In plants grown in an unheated greenhouse during January, February and March, the first internode was scarcely visible, but in plants raised in the greenhouse during the warmer summer months it elongated to a length of 3–4 mm.

Summer turnip rape (B. campestris *L. forma* annua).
(Plate XXII, fig. 7.)

Cotyledons. In seedlings grown out of doors and in the greenhouse, the cotyledons are similar to those of the white-fleshed turnip. In seedlings raised in the dark the cotyledons are a deep yellow colour like those of the yellow-fleshed turnip.

Hypocotyl. The hypocotyl is greenish violet, shading to white below the surface of the soil.

First leaf. The lamina is very variable in shape; common forms are:

(1) Ovate (*circa* 25 mm. long by 14 mm. broad), with a fairly large terminal tooth, and four pairs of lateral teeth.

(2) Ovate (*circa* 32 mm. long by 20 mm. broad), widest across the middle with a small terminal tooth, and four pairs of lateral teeth.

(3) Ovate (*circa* 26 mm. long by 15 mm. broad), with a small terminal tooth and four pairs of rounded lateral teeth.

(4) Lyrate (*circa* 23 mm. long by 13 mm. broad), with a large, slightly toothed terminal lobe, and two small lateral lobes.

The first leaves are almost the same colour as those of winter turnip rape. The veins are prominent; in some plants they are pale green, in others they contain a small amount of violet pigment.

There are many stiff white hairs on the upper surface, around the margin of the lamina, and on the veins on the lower surface.

The petiole is pale green to greenish violet, and clothed with a variable number of stiff white hairs, especially on the lower convex surface.

First internode. This is not visible in 90 per cent. of plants grown out of doors; in a few plants it is elongated up to about 2 mm., the same colour as the hypocotyl and covered with stiff white hairs.

In plants grown in an unheated greenhouse during January, February and March, the first internode is not visible, but in a large number of plants raised in the greenhouse in April and May it is 3–4 mm. long.

DISCUSSION.

From the foregoing observations it will be clear that there are no absolutely constant points of difference between the seedlings of swede turnips and swede rapes; similarly, no constant morphological characters are found between the bulb-forming turnips and their corresponding leafy turnip rapes.

When dealing with winter swede rape (*B. Napus* L. forma *oleifera*), Percival(7) states that "both seeds and seedlings are identical in appearance with those of swede turnips." I understand that this statement refers to seedlings in the cotyledon stage before the first rough leaf has developed, in which stage of development swedes and their corresponding rapes are indistinguishable.

With regard to the shape of the first foliage leaf, the predominant form in the yellow-fleshed swede is roundish, widest across the middle, with a small terminal tooth, and a large number of irregular, small pointed lateral teeth. There are, however, some seedlings of the yellow-fleshed swede which are widest about one-third of the distance below the apex, and have a large terminal tooth and few lateral teeth, such leaves very closely resembling those of winter swede rape.

When a large number of seedlings of the swede rape and swede turnip are viewed in mass, a fairly reliable guess may be made concerning them, but it is not possible to distinguish with certainty individual seedlings of either group.

In a large proportion of the seedlings of white-fleshed swede turnip the first foliage leaf is indistinguishable from those of swede rape. The first foliage leaves of summer swede rape (*B. Napus* L. forma *annua*) are identical in appearance with those of winter swede rape.

Regarding the second character, namely, the relative length of the first internode in swede turnips and rapes, Gorman and Lafferty(1) state: "In examining the Swede seedlings eighteen days after the seeds were sown, it was noted that the petioles of the cotyledons, and those of the first foliage leaves appeared to arise at approximately the same level on the stem, but that in the case of the rape seedlings a distinct internode varying in length from 2 to 5 mm. was invariably present." The seeds were sown early in June in an unheated, well-lighted greenhouse, and apparently no tests were made during the colder months.

The present observations were made on successive batches of seedlings sown at approximately monthly intervals throughout the year in an unheated greenhouse. Contrary to the observations and conclusions of Gorman and Lafferty (1) I find that during the winter months the seedlings of all forms of swede turnips, common turnips, and rapes, show no elongation of the first internode. Even when the seedlings were planted out and kept under observation until the fifth or sixth foliage leaf had expanded, the first internode always remained quite invisible.

During the warmer months, however, seedlings of summer swede rape and winter swede rape showed elongation of the first internode fairly early, but a large proportion of the seedlings of the white-fleshed swede and a number of those of the yellow-fleshed swede also showed elongation of the first internode very shortly afterwards.

Although the first internode of rape elongates more readily than that of the swede turnip, the presence of a clearly extended internode is not conclusive evidence that the seedling in question is one of rape; nor does the absence of a clearly extended internode prove the seedling to be one of the swede turnip.

When seedlings of the yellow-fleshed swede are grown under ordinary farm conditions, the statement that the first internode does not elongate is not correct, for when seedlings of yellow-fleshed swede were examined in the field just before thinning many were found to have their first internodes much elongated, up to 5 or 6 mm. at this stage of growth.

Rape seedlings grown in the open during summer usually show an elongated first internode, but there are always some plants in which the first internode remains unextended.

Towards the end of June two rows of seedlings of the white-fleshed swede, two of yellow-fleshed swede, two of winter swede rape, and two of summer swede rape were planted out, one row of each consisting of plants with elongated first internodes, the other of plants without elongated first internodes. In a few weeks after planting out, it was impossible to detect any difference between plants which had exhibited an elongated first internode and plants in which the first internode had remained short.

The plants were allowed to develop; those of summer swede rape flowered in the autumn of the same year, while those of the winter swede rape, white-fleshed swede, and yellow-fleshed swede flowered in the following spring.

With reference to the turnip "group," white-fleshed turnip, yellow-fleshed turnip, winter turnip rape and summer turnip rape, the shape of the first foliage leaf seems to be of no value in the identification of the respective seedlings, as the form varies so much in each of these Brassicas. The first foliage leaves, however, of the turnip rapes are darker in colour than those of the bulbing turnips, and this character may be of some value in attempting to distinguish between their seedlings.

Only very few plants of the seedlings of turnip and turnip rape show any sign of elongation of the first internode when grown in the open.

When grown fairly close together in the greenhouse during summer, a considerable number of plants have an elongated first internode, but the tendency of the first internode to elongate is just as great in turnips as it is in turnip rapes, and the character is of no diagnostic value in distinguishing them.

The cotyledons of seedlings of white-fleshed swede, white-fleshed turnip and winter and summer swede rapes when grown in the dark are a pale yellow tint, while those of yellow-fleshed swede, yellow-fleshed turnip and winter and summer turnip rapes are of a deep yellow colour.

SUMMARY.

A detailed study was made of the morphological characters of the seedlings of some cultivated forms of *Brassica*, namely, white-fleshed and yellow-fleshed swede turnips, winter swede and summer swede rapes, white-fleshed and yellow-fleshed common turnips, and winter and summer turnip rapes.

It is shown that there are no constant points of difference between the seedlings of swede turnips and swede rapes, nor between the seedlings of the bulb-forming turnips and their corresponding leafy turnip rapes.

These investigations were carried out at the Agricultural Botanic Garden and in the Department of Agricultural Botany, University of Reading, under the direction of Prof. Percival, to whom I desire to express my cordial thanks for suggestive criticism and assistance with the illustrations.

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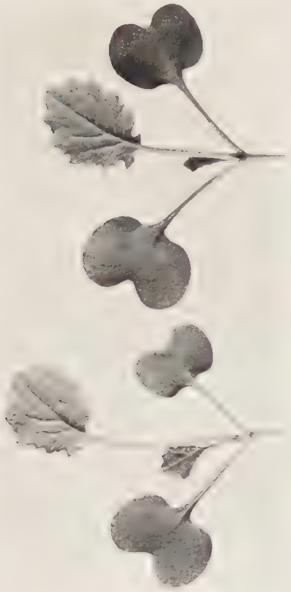

Fig. 1.

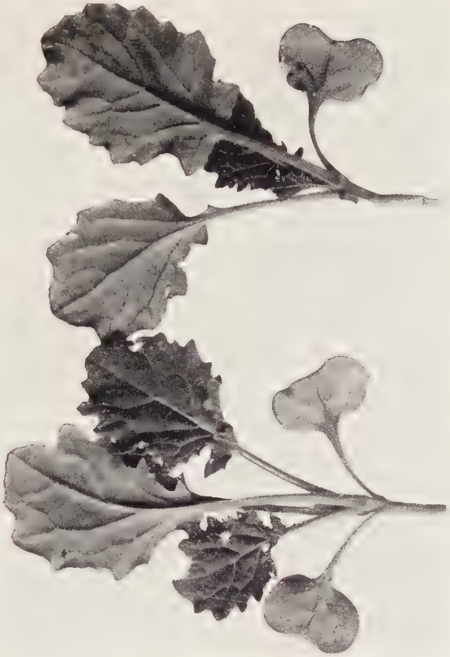

Fig. 3.

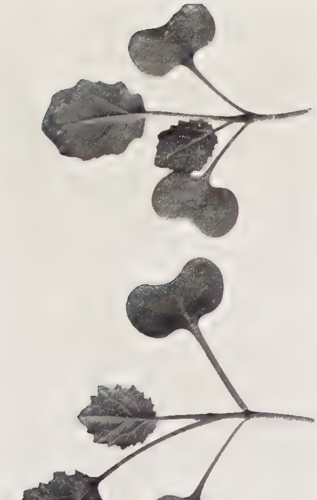

Fig. 4.

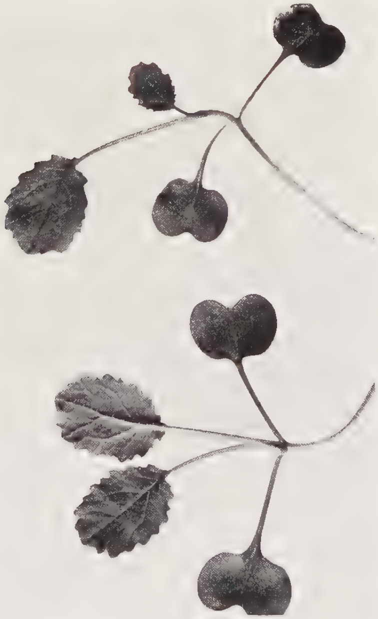

Fig. 2.

ROBSON.—The Seedling Characters of Some Cultivated *Brassicae* (pp. 418–429).

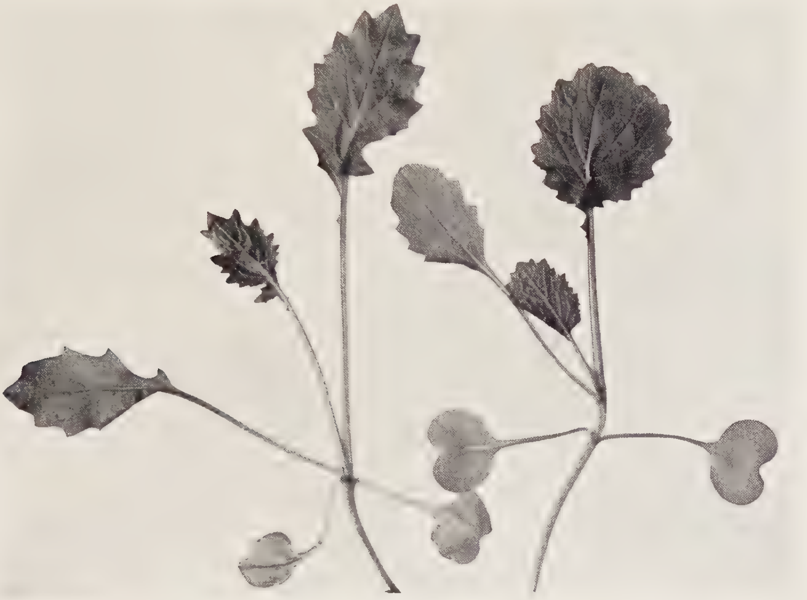

Fig. 5.

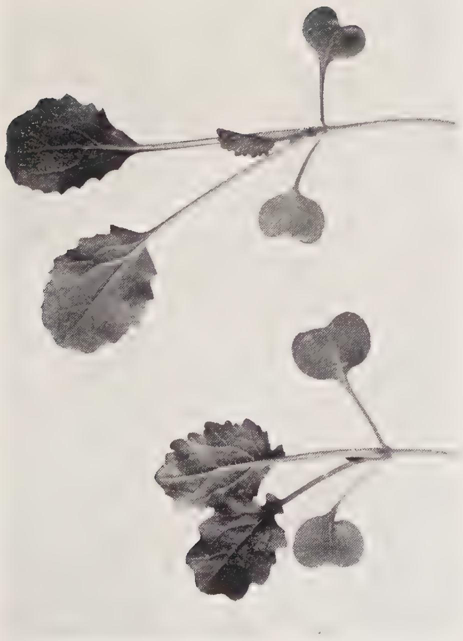

Fig. 6.

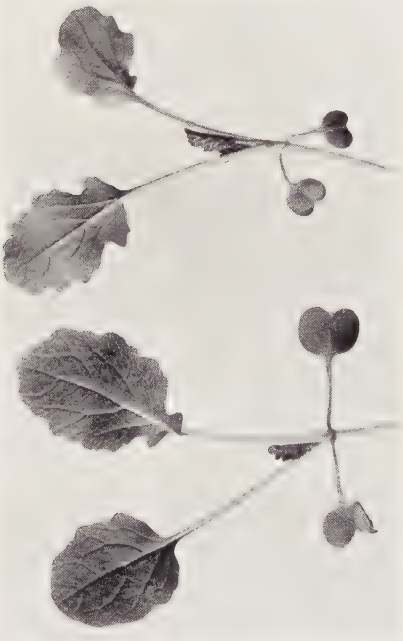

Fig. 7.

ROBSON.—The Seedling Characters of Some Cultivated *Brassicae* (pp. 418–429).

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EXPLANATION OF PLATES XXI AND XXII.

Each figure shows two seedlings of the same species grown under the same conditions, one with an elongated first internode, the other in which the first internode has remained unextended.

PLATE XXI.

Fig. 1. White-fleshed swede turnip.
Fig. 2. Yellow-fleshed swede turnip.
Fig. 3. Winter or broad-leaved swede rape.
Fig. 4. Summer swede rape.

PLATE XXII.

Fig. 5. White-fleshed common turnip.
Fig. 6. Winter turnip rape.
Fig. 7. Summer turnip rape.

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EXPERIMENTS BEARING ON THE NATURE OF INTRACELLULAR INCLUSIONS IN PLANT VIRUS DISEASES

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(With Plates XXIII–XXV.)

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INTRODUCTION.

Intracellular inclusions in virus diseases.

Some thirty years ago Iwanowski first discovered the curious cytological effects which may be induced by infection of a plant with a virus disease (9). He then described and figured the peculiar amoeboid bodies which he found in the cells of tobacco with mosaic disease. Since that time the presence of similar intracellular inclusions has been reported in many plants and animals attacked by virus diseases and consequently considerable attention has been attracted to them. It is unnecessary here to discuss the literature as it has frequently been summarised (5, 8, 17), and a full list of papers is given by K. M. Smith (18). Some virus diseases appear to be invariably characterised by these bodies whilst other viruses have no visible effect on the cells of the host. For example, inclusions are always found in the cells of plants infected with tobacco mosaic (5), dahlia mosaic (6) and sugar-cane mosaic (10), but could never be found after infection with cucumber mosaic or petunia mosaic (8). Such intracellular inclusions had never been found in healthy plants nor in plants infected with any disease other than a virus. The bodies are protein in nature and show certain apparent similarities in form and behaviour to amoeboid organisms. In fact, the view was frequently advanced that the bodies were actually organisms, that they were the etiological agents of the disease. However, other workers held the view that they are inanimate, being merely pathological effects.

Aucuba mosaic disease.

Some recent work on the intracellular inclusions in solanaceous plants infected with aucuba mosaic disease of tomato, has lent considerable support to this latter conception of their nature (14). By watching under the microscope living cells of infected plants, continually from the time of inoculation until the maturing of the inclusion bodies, these bodies were seen to be formed by the aggregation and fusion of minute particles of protein which appeared in the cytoplasm of the host a few days after infection. An increasing activity of the cell contents is the first effect of the virus to become apparent microscopically. The cytoplasm becomes more conspicuous, it seems to increase in volume and it flows around and about the cell with increasing rapidity. Then minute yellowish particles appear in the cytoplasm which, as it flows, carries them passively about the cell. If these particles are brought together by converging streams of plasm, they fuse. Gradually the particles increase in size, then they aggregate into a few large masses. These masses unite together until practically all the protein material is contained within a single loosely packed, irregularly shaped body. It soon loses its irregular form, becoming more compact as it assumes a roughly spherical outline. It may become vacuolate (Plate XXIV, fig. 4). After some weeks this body crystallises out and the crystals are dissolved into the cell sap.

These observations, whilst not precluding the idea that the virus may be contained within the body, indicate that the main bulk of the body is formed from the cytoplasm of the host in response to the stimulus of the virus. They further suggest that the body is essentially a coagulated portion of the cytoplasm.

The mode of formation of bodies due to infection with aucuba mosaic disease was watched in a number of hosts. A few differences were noted in the mature body. In *Solanum nodiflorum*, *S. nigrum* and *S. lycopersicum* the body almost always becomes smoothly rounded. In *Nicotiana tabacum*, *N. glauca* and *N. rustica* the body frequently fails to round off, and in *Hyoscyamus niger* it very seldom does so but persists as a large irregularly shaped granular mass. In the above species, infection is systemic. In *Nicotiana glutinosa*, where local lesions are produced by this virus, no intracellular inclusions could be found.

A number of different hosts infected with various virus diseases have been examined cytologically from time to time. No results have been published, but two examples might, with advantage, be briefly summarised here.

Hy. III disease.

Of the several new virus diseases of *Hyoscyamus niger* recently described (7), Hy. II and Hy. IV produce no inclusion bodies. However, Hy. III regularly induces the production of abnormal structures within the cells of the host plant. For the study of the cytological effects, hosts from the same range that was used with aucuba mosaic were tested and a somewhat similar response was obtained suggesting that, although the production of the body is determined by the virus, its character may be slightly modified by the host. Of the three species of *Solanum*, *S. nodiflorum* and *S. nigrum* proved to be immune to Hy. III, but in *S. lycopersicum* systemic infection is accompanied by the production of large spherical cellular inclusions very similar to those induced by aucuba mosaic disease. In both *Nicotiana tabacum* and *Hyoscyamus niger* the response to the two diseases is similar. In the former there is a tendency for the bodies to remain irregular in form and in the latter they very seldom round off. The bodies induced by Hy. III in these three hosts are formed in precisely the same way as those due to aucuba mosaic infection. The first microscopic effect to become obvious is the accelerated streaming of the cytoplasm in which tiny protein particles soon appear. These ultimately all coalesce to form a single large body (Plate XXIII, fig. 3).

Tobacco mosaic disease.

Much work has been done on the cytology of mosaic disease of tobacco, Goldstein in particular having made a very careful and detailed study (5). Her description of X-bodies[1] would, except in a few details, appear to be equally applicable to the inclusion bodies formed by aucuba mosaic but Goldstein is convinced that the X-bodies "are not products of the metabolism of diseased cells." An extensive study has therefore been made of fixed and living material of plants infected with tobacco mosaic in an attempt to discover any correlation between the X-bodies of tobacco mosaic and the inclusions formed by aucuba mosaic. The range of hosts used included *Nicotiana tabacum*, *Solanum nodiflorum*, *S. nigrum* and *S. lycopersicum*, all of which yielded similar results. The virus used was Johnson's tobacco virus I.

Seen in fixed material, the inclusions induced by tobacco and aucuba mosaic viruses are not unlike, but when seen side by side and in living cells they appear strikingly different. However, their chemical reactions are alike, so that it is not easy to define adequately the differences which seem to lie in the size and consistency of the bodies. X-bodies of tobacco mosaic are usually much smaller than the inclusions of aucuba mosaic disease. They are also far less granular, appearing to have rather the same consistency as the cytoplasm of the cells containing them. In fact, they appear very like a portion of cytoplasm which, although it has no apparent limiting membrane, is able to retain intact its complement of chondriosomes and oil globules. Goldstein described a membrane, but the writer has been unable to observe this in any virus inclusion bodies examined. An apparent membrane is seen in some photographs, but this is merely an optical effect which when observed visually can be eliminated by refocussing the microscope. The X-body is continually carried about the cell in the cytoplasmic stream. It may become distorted through the buffetting of the flowing plasm just as the nucleus may become temporarily misshapen, but, like the nucleus, it appears always to retain its individuality (Plate XXIV, fig. 1). Many infected plants were examined both before and after the appearance of X-bodies, but no evidence could be obtained as to their origin. It was evident that they were not formed by fusion of particles as are aucuba mosaic bodies, but their origin remained obscure, as no transitional stage between an apparently normal cell and one containing an X-body could be found. After persisting for some weeks, these bodies disappear as suddenly as they arise. They do not crystallise as do the bodies of aucuba mosaic disease.

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[1] The use of the term "X-body" is, in this paper, confined to the amoeboid intracellular inclusions of tobacco mosaic originally so designated by Goldstein.

Associated with tobacco mosaic disease is regularly found a second type of inclusion which at first seems to be similar to many of the crystalline bodies found in healthy plants. This is the striate material (Plate XXIV, fig. 1). It consists of large polygonal plates, either hexagonal or irregular, when they may have been formed by the fusion side by side of smaller hexagonal plates. In side view these plates appear rectangular and show faint lines of striation. On treatment with fixatives the striation becomes more distinct. This striate material persists after the disappearance of the X-body. If plants infected with tobacco mosaic disease are kept for a prolonged period, the vivid dark green mottle is lost from the leaves which tend to develop a yellow blotching. At the same time, it is found that the polygonal crystals in the cells are coalescing, ultimately they are all contained within a single mass which becomes rounded and seems to be in all details like the bodies due to aucuba mosaic disease (Plate XXIV, fig. 3).

ATTEMPTS TO INDUCE CHANGES IN THE COLLOIDS OF THE PLANT CELL.

In considering the nature of the intracellular inclusion bodies, the fact that they are confined to plants and animals infected with virus diseases would appear to be peculiarly significant. However, the mode of formation of the inclusions due to aucuba mosaic virus led almost to the conviction that certain of the bodies are merely coagulation products. Thereby was suggested the possibility that the production of morphologically similar bodies in otherwise normal healthy tissue might be induced by judicious treatment with coagulating agents. Some attempts have therefore been made to determine whether, by treating plants with colloidal reagents:

(*a*) similar abnormal bodies could be induced in the cells of uninfected plants, and

(*b*) the reverse reaction could be induced in diseased cells either by inhibiting the formation of bodies or by artificially dispersing them after formation.

A considerable amount of work of a similar nature on animal tissues has been carried out by Bancroft and his co-workers (1,2,4). For instance, certain mental disorders appear to be due to abnormal variations in the colloids of the nervous tissue. Variations are in degree of dispersion only and hence cannot be demonstrated by any histological technique. By use of colloidal reagents, it is possible to induce symptoms in the normal nervous system not unlike many of those of insanity. Certain functional disorders, due to agglomeration of the colloids of the central nervous system, can be overcome by use of peptising agents as can also the effects of certain drugs. The action of anaesthetics is due to reversible coagulation, and this seems to be true of anaesthesia of both animals and plants (3).

To parallel the phenomena produced by virus diseases involves the production of changes very much greater in degree than anything previously brought about experimentally in the living cell. Further, it was desired to coagulate only a fraction of the cytoplasm, allowing the rest to maintain the normal processes of life. There was available very little experimental data on which to base the work so that only "hit or miss" methods could be employed. A large number of substances are known to coagulate the proteins of the cell. In spite of the vast amount which has been written about the physical and chemical properties of living matter, its constitution is still largely enigmatical. Whilst this remains so, the action of any specific reagent cannot be properly understood or explained. Investigators are handicapped both by the minute dimensions of the cell and by the difficulty of preserving the protoplasm in its normal living state whilst under observation and experimentation. In the case of plants, further difficulties are introduced by the impenetrable nature of the cell wall. Thus, existing knowledge is insufficient to indicate the use of any one series of reagents in preference to any other for the present purpose. It was impossible to try all the substances which suggested themselves, and at first a selection could be made only at random. A number of mineral and organic substances, many of which were known to precipitate cytoplasm, were chosen. During the course of the work, the results obtained by the use of one reagent often suggested the employment of other substances which normally show similar reactions. Occasion arose to obtain spectrographic analyses of certain treated plants for comparison with normal and virus diseased ones. Incidentally, differences were found in the amounts of certain elements present in the specimens of *Solanum nodiflorum* infected with aucuba mosaic and in the normal plants. In each case leaves of some half dozen plants were ashed together, but only one series of analyses was made so the differences in the proportions of these elements might be due to individual variation. This observation accounted for the inclusion of salts of aluminium and nickel, among the reagents used.

A further difficulty was encountered in deciding the concentration of the reagent to be used, especially as many of the substances chosen were known to be highly toxic. The ideal method would be the employment of a large range of concentrations of each reagent. This would have occupied much time and glasshouse space and would, of necessity, have greatly reduced the number of reagents it was possible to employ. It was felt that useful results were more likely to accrue by testing a large number of reagents rather scantily than by the trial of a few reagents in far greater detail. Therefore, in the first instance, one purely arbitrary strength of solution of each substance was selected. If this solution proved toxic, then weaker ones were tried. If the results gave any promise, then new experiments were set up with weaker or stronger solutions as appeared expedient. Alternatively, in some cases the number of doses given was varied.

A short note has already been published on this work (16); a more detailed description now follows.

EXPERIMENTAL.

Attempts to simulate virus inclusion bodies in uninfected cells.

Material.

Solanaceous hosts have proved very favourable subjects for the study of the cytological effects of virus diseases by vital methods. Where virus inclusion bodies are found, they are usually most evident in the tegumentary tissues of the leaves. If the latter bear protective hairs then the bodies are usually very abundant in them. Trichomes can be examined microscopically with great simplicity whilst still living. Several of the Solanaceae have hairy leaves and their cells are usually free from ergastic substances which might complicate a study of abnormal inclusions. *Solanum nodiflorum* is particularly useful, for, not only are the cells very clean and clear, but the whole trichome is invested by a thick cell wall. For examination, a strip is cut from the edge of the leaf and is mounted on a slide in water or in isotonic sugar solution. The thick wall delays penetration by the mounting medium for some hours, so that the same cell can be kept under observation for prolonged periods.

S. nodiflorum was used in every experiment in this study. *S. lycopersicum*, *Nicotiana tabacum* and *Hyoscyamus niger* were used for comparison from time to time.

All observations were made on living material. The cells of trichomes were always extensively examined. Occasionally mounts were made of the epidermis by stripping it from the leaf and mounting in an isotonic solution. Hand sections were also occasionally used. In such work no great reliance could be placed on observations on epidermal mounts or hand sections as the cells very soon die and also the mounting medium penetrates rapidly, bringing about abnormal conditions almost immediately. These methods were used only for comparison with the observations made on unsectioned material.

Methods.

Owing to differences in general structure and largely to the nature of the cell wall, the use of vital methods in cytological study is notoriously beset by many more difficulties in plants than in animals. In the work on anaesthesia in plants the usual method of approach is through spraying of the plants. This method was tried but with not much success. Some coagulation, visible microscopically, was obtained, but it was not possible to increase the dose to give greater coagulation without injury to the tissues. More drastic measures had to be employed, the following methods being used.

(1) *Soil treatment.*

The plants were grown in our usual compost: this is grass turves and stable manure stacked for 12–18 months before use, when some sand is added. Pots of approximately 6 in. diameter (32's) were used. Instead of keeping the soil moist with water in the ordinary way, a dilute solution of the appropriate reagent was employed, the pots being stood in saucers to prevent loss of the reagent. Stock solutions of the substances to be used were made and stored in aspirators. Where possible the solutions were of such a strength that 10 c.c. could be added to 1 litre of water at the time of use. Water is laid on to each chamber of the glasshouse. In each a large tank is kept always full so that the water used for the plants is of an equable temperature. Water was taken from this tank for diluting the solutions. In these experiments plants were usually treated in batches of three or multiples of three. Usually 1 litre of the dilute fluid was divided roughly between three pots each time they received treatment. The solutions were made up accurately but no great care was taken to divide the litre exactly as, even if it were known precisely how much of the reagent each pot received, it would be no reliable guide to the amount the plant actually takes up. From time to time throughout treatment, and for some days after the last dose was given, small portions of leaves were taken from the plant and their cells were examined. Usually the treatment was continued until each plant had received a litre of the dilute fluid, but this was often varied as circumstances demanded. Control plants were kept in saucers and received water on each occasion that the experimental plants were treated.

This method proved to be the neatest and simplest. It has the great disadvantage that the reagent may be precipitated by other substances in the soil and consequently will never be absorbed by the plant. The effect of every reagent was tested by this method but the following alternatives were used from time to time.

(2) *Cut shoots.*

Young axillary shoots were removed from a plant, the cut being made under water to prevent the entrance of air into the plant tissues. The cut end of the shoot was then inserted into a small conical flask containing a dilute solution of the reagent. The solutions used were 1/5 to 1/50 of the strength used in method I. Cells were examined microscopically from time to time during the experiment.

(3) *Suction through cut petiole.*

The petiole of a leaf was cut under water so as to remove the lamina. The cut end of the petiole was immediately immersed in a small tube containing a dilute solution of the reagents. The solution was drawn back through the petiole into the vascular system of the stem and thence to other tissues.

This method was not a very satisfactory one. If a relatively strong solution were used it killed the tissue of the petiole so that the cells became plugged and absorption ceased. If weaker solutions were used they became very dilute before reaching the tegumentary tissues on which most of the observations were made. The solutions used varied from 1/10 to 1/100 of the strength of those used in method I.

(4) *Injection.*

A very dilute solution was injected into the lamina of a leaf by means of a hypodermic syringe. It was found simplest to make the puncture through the lower epidermis at the juncture of two fine veins.

The solutions used were about 1/10 to 1/100 of the strength of those used in method I.

No positive results were obtained by injection into the petiole, due probably to the difficulty of suitably adjusting the strength of the solution.

Results.

Formalin.

Method I. 1 per cent. Toxic to all plants.

0·1 per cent. *Hyoscyamus niger*, lower leaves flagged, upper ones became crinkled. In *Hyoscyamus niger*, *Solanum nodiflorum* and *Nicotiana tabacum*, after two days the cytoplasm was found to be streaming very rapidly. This continued for some days.

0·05 per cent. *Hyoscyamus niger* leaves showed slight crinkling. Microscopic effects same as with 0·1 per cent. solution in all test plants used.

Method IV. 0·005 per cent. Cytoplasm of *Solanum nodiflorum* flowed very rapidly in cells neighbouring seat of injection.

Chloroform.

Method I. 1 per cent. *Solanum nodiflorum*, *S. lycopersicum* and *Nicotiana tabacum*. The cytoplasm was found to be streaming very rapidly. It also became very conspicuous and later whole strands seemed to become more viscous, but there was no indication of any localised coagulation (Plate XXIII, fig. 4).

2 per cent. This solution was toxic to *Solanum nodiflorum*, the lower leaves dying off rapidly. Other hosts behaved as with the weaker solution.

Ethyl alcohol.

Method I. 2 per cent. In the hairs of *Solanum nodiflorum*, *Nicotiana tabacum* and *Hyoscyamus niger*, the cytoplasm streamed with increasing rapidity, also becoming very conspicuous.

4 per cent. In the same range of plants, coagulation of the cytoplasmic strands followed accelerated streaming.

Method II. 0·5 per cent. Results as with method I.

Method IV. 0·1, 0·5 per cent. *Solanum lycopersicum*. Increased rate of streaming in neighbouring hair cells.

Urea.

Method I. 0·1 per cent. (pH=7). *Solanum nodiflorum*. Leaves assumed a very dark green colour. There was accelerated streaming of the cytoplasm which became more conspicuous. Treatment was continued in the same plants until 2 litres of solution had been added to each. No further abnormalities resulted.

Acetic acid.

Method I. 0·1 per cent. *Solanum nodiflorum, Nicotiana tabacum, Hyoscyamus niger.* After 12 hours, coagulation was observed. Plants wilted and died immediately.

0·02 per cent. Same hosts. Accelerated streaming and increased conspicuousness of cytoplasm.

Method II. 0·002 per cent. *Solanum nodiflorum.* Accelerated cytoplasmic streaming in the hair cells.

Lactic acid.

Method I. 0·1 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* The cytoplasm became very conspicuous, it increased in bulk and rapidity of movement. This was especially noticeable in *H. niger,* where the movement contrasted strikingly with the usually rather sluggish streaming of the plasm in the normal plant.

0·2 per cent. Same hosts. As before, the cytoplasm increased in bulk and flowed more rapidly about the cell. Occasionally a hair cell was found in which one small portion of the cytoplasm had become slightly more conspicuous than the rest (Plate XXIV, fig. 2). As the plasm flowed about the cell, this small portion was carried along with it. No limiting membrane could be observed, but this little globule of cytoplasm retained its own identity even when buffeted by converging streams of plasm so as to become misshapen. These bodies were in appearance somewhat similar to the X-bodies of tobacco mosaic virus. They were, however, comparatively rare, and cells containing them difficult to find. When found, these bodies could usually be observed for an hour or so. Then they were suddenly lost to view, having apparently been re-absorbed into the general mass of the plasm.

In all, thirteen *Solanum nodiflorum,* three *S. lycopersicum* and two *Hyoscyamus niger* received this treatment of 1 litre of 0·2 per cent. lactic. The bodies were found in nine of the *Solanum nodiflorum* and in one tomato, but they were not found in *Hyoscyamus niger.* 0·4 per cent. solution was tried on three *Solanum nodiflorum.* No bodies could be found. It is possible that they did not form, as the cytoplasm seemed to become generally more viscous.

Method III. 0·01 per cent. *Solanum nodiflorum.* Petiole shrivelled. 0·001 per cent. *S. nodiflorum.* Accelerated streaming of the cytoplasm was noticed in some of the cells of the leaves above the one used to introduce the solution. No bodies could be found.

Method IV. 0·001 per cent. *Solanum nodiflorum, Nicotiana tabacum.*

In some hairs immediately surrounding the puncture, the cytoplasm seemed to have become generally coagulated. It was very conspicuous and movement was very sluggish.

Picric acid.

Method I. 0·025 per cent. *Solanum nodiflorum, S. lycopersicum* and *Hyoscyamus niger.* Yellow spotting of leaves.

0·0125 per cent. Same hosts. There are often a few chloroplasts in the hairs of normal plants. These seemed to increase in number. There was very little difference from usual in the appearance of the cytoplasm.

Sodium pyruvate.

Method I. 0·1, 0·2 per cent. *Solanum nodiflorum.* There was no visible effect on the cells.

Nicotine.

Method I. 0·001 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* A mottle was often produced on two or three leaves of the plant. Microscopically, these leaves did not differ at all from those which retained their normal appearance. The rate of streaming of the cytoplasm was greatly increased. Minute hyaline spheres, which seemed to have a slightly different consistency from the rest of the cytoplasm, made their appearance in it and were carried rapidly about the cell.

0·005 per cent. *Solanum nodiflorum.* There appeared to be considerable coagulation in the cytoplasm.

Hyoscyamus extract.

Method I. 0·002, 0·005 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* No effects were visible in the cells.

Cannabis extract.

Method I. 0·005, 0·01 per cent. *Solanum nodiflorum.* No microscopic effects were visible.

Strychnine.

Method I. 0·01, 0·05 per cent. *Solanum nodiflorum.* The usual initial stimulation of the cytoplasm preceded a slight degree of general coagulation.

Method IV. 0·005 per cent. *Solanum nodiflorum.* It was possible to induce some slight thickening of the cytoplasm in the cells of hairs near the puncture.

Iodine.

Method I. 0·05 per cent. Toxic.

0·01 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* Apart from an initial stimulation of the cytoplasm, no cytological effects were visible.

Copper sulphate.

Method I. 0·01 per cent. (pH=5·4). *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* Stimulation of the cytoplasm was followed by a slight apparent increase in viscosity.

Barium chloride.

Method I. 0·1 per cent. *Solanum nodiflorum, S. lycopersicum, Nicotiana tabacum.* No effects visible microscopically.

Zinc chloride.

Method I. 0·01 per cent. *Solanum nodiflorum.* No microscopically visible effects.

Mercuric chloride.

Method I. 0·01, 0·02 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* Some slight stimulation of the plasm was followed by an increase in viscosity.

Iron alum (ferrous ammonium sulphate).

Method I. 0·1 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* Plants became very brittle. The streaming of the cytoplasm was increased. Treatment was continued until each plant had received 2 litres of solution. Some coagulation was then produced in the cells of the hairs.

Aluminium citrate.

Method I. 0·05 per cent. *Solanum nodiflorum.* No effect was visible in the cells of the hairs.

Lead nitrate.

Method I. 0·05, 0·1 per cent. *Solanum nodiflorum.* Early stimulation of the streaming cytoplasm was the only visible effect produced in the plant cells.

Nickel chloride.

Method I. 0·1 per cent. *Solanum nodiflorum.* The cytoplasm was stimulated to stream more rapidly. Later, some slight coagulation was brought about in the cells of the leaf hairs.

Potassium arsenate.

Method I. 0·01, 0·02 per cent. *Solanum nodiflorum, S. lycopersicum, Hyoscyamus niger.* The cytoplasm appeared to increase in bulk in all the plants. In *Solanum nodiflorum,* small particles were seen in the plasm. Later, some degree of coagulation of the cytoplasm occurred.

Ammonium molybdate.

Method I. 0·1 per cent. ($p\mathrm{H}=4{\cdot}4$). Initial observations were made on *Solanum nodiflorum, S. lycopersicum, Nicotiana tabacum* and *Hyoscyamus niger,* but later work was confined to the first-named species. This reagent produced very striking macro- and microscopic effects on all four hosts. It caused stunting of the plants and the leaves became bleached. These two symptoms varied in degree with the quantity of reagent received by the plant. One litre of 0·1 per cent. solution was sufficient to bleach completely all the leaves of a *Solanum nodiflorum* plant which was about 9 in. high at the time of treatment. $1\frac{1}{3}$ litres of this solution was completely toxic. Microscopic effects and slight macroscopic symptoms were produced by a litre of 0·025 per cent. solution. After treatment ceased the plants commenced to grow out of the effects. Yellowish green leaves were produced and sometimes mottled ones. This mottle bore very little resemblance to one due to virus infection, but it was typical of the presence of some toxic substance. Plants which had been treated never became entirely normal again. The lamina of the leaf of a normal plant is about 4 in. long, that of a treated plant seldom exceeded half this length. The whole habit of the plant can be changed by this treatment. *S. nodiflorum,* as grown in our glasshouses, produces first a single upright stem. When this is about 2 ft. high, small axillary branches grow out. Treated plants branched profusely, the secondaries being indistinguishable from the main stem. The internodes were much shorter than in the normal (Plate XXV, fig. 1). About two months after treatment, the plants bore a strong superficial resemblance to *S. nigrum.* They had the same trailing habit. The main difference lay in the form of the leaves, those of treated *S. nodiflorum* plants retained their entire margin whilst those of *S. nigrum* are coarsely toothed.

A *S. nodiflorum* plant treated in September was kept over the winter. In February, a few leaves showed a mottle resembling a virus symptom. It was thought that the plant had become accidentally infected, but it was impossible to transmit the mottle by needle inoculation. Attempts have been made to produce this mottle again. Two plants, one six and one eight months after treatment, have shown isolated mottled leaves

(Plate XXV, fig. 2). This mottle could not be transmitted by needle inoculation into *S. nodiflorum* or *Hyoscyamus niger* nor by rubbing leaves of *Nicotiana glutinosa* with juice from an affected leaf. The material obtained was insufficient to make any other transmission tests possible. It seems unlikely that the mottle was due to an accidental infection with a virus, for if a virus were present the symptoms would probably have been more general.

Ammonium molybdate treatment produced curious changes in the cells of the plants. Soon after treatment began the cytoplasm was seen to be moving very rapidly around and about the cell and it seemed to increase in bulk (cf. Plate XXIII, fig. 4). Minute particles appeared in the plasm which carried them about the cell. The particles gradually agglomerated until a single large mass of them was formed. This mass persisted in the cell as a yellowish granular body (Plate XXIV, figs. 5 and 6). These bodies were very abundant in the tissues. They were present in practically every hair cell, were abundant in the epidermis and also occurred in the palisade tissue. The plants still continued to produce them in new tissue, six months after treatment ceased.

Morphologically these bodies appeared exactly similar to those produced by aucuba mosaic and Hy. III diseases and they were formed in a precisely similar way. It will, however, be seen that they are of a different chemical nature.

It was suggested, as the salt was applied to the roots of the plant, that it might not reach the shoot and that the formation of inclusion bodies was due to some secondary effect. At this time no satisfactory microchemical tests had been devised and hence some spectrographic analyses were made by Dr S. Judd Lewis. The molybdenum content of normal leaves, of leaves from plants infected with aucuba mosaic disease and from plants treated with ammonium molybdate were analysed. Molybdenum was found to be abundant in the leaves of treated plants.

It has been generally thought that molybdenum was not usually present in plant ash (13). In view of recent work by ter Meulen (11), who has examined coal ash and also the ashes of a number of widely different plants and has invariably demonstrated the presence of molybdenum, and of the work of Roach (12), who is determining the exact significance of this element in the development of certain apple grafts, it is of interest to note that this element was also present in both normal and virus diseased plants of *Solanum nodiflorum*. Healthy leaves contained approximately 0·015 per cent. molybdenum relative to the calcium content and the diseased leaves showed 0·011 per cent. The calcium content of the ash was not accurately determined, but it is about 10 per cent., so that the amount of molybdenum present represents a little over 0·001 per cent. of the ash.

Microchemical methods were later devised to test these inclusion bodies. They are thought to contain a certain amount of protein, as a pink coloration is produced in them by warming a strip of living tissue with Millon's reagent; with ammonia and nitric acid, they assume a deeper yellow colour. The thickness of the cell wall often prevents penetration by less strong reagents so that difficulty is experienced in obtaining satisfactory results. A method of micro-incineration is therefore being developed. The apparatus at present available is somewhat crude, an ordinary electric furnace with a refractory clay lining being used. Although there may be some cracking of the thicker cell walls, sufficiently good preparations have been obtained. Paraffin sections could not be used owing to the difficulty of finding fixatives which would neither distort unduly, nor would dissolve out, nor add, mineral substances. Frozen sections and hand sections and also strips of epidermis torn from the leaves were heated until a temperature of 550° C. was attained. This took 60 minutes. On cooling, each preparation was covered with a $2 \times \frac{7}{8}$ in. glass slip which was sealed with paraffin wax. After examination with dark ground illumination when the inclusions were seen to be of a blue colour, the sections were used for chemical tests. The wax was scraped from one end of the slip and a bubble of reagent was placed on the slide at this end. At the far end of the slip a hole was made in the wax to allow escape of air and entrance of the solution. This hole was small to prevent a sudden flooding of the slide which might displace the ash. The section was watched as the reagent passed over it. In this way the inclusion bodies were shown to contain molybdenum as they invariably gave a red coloration with a xanthate solution.

The molybdenum evidently enters into combination with some of the mineral or organic constituents of the cell, but the nature of the compound which is formed is at present unknown. The presence of phosphorus was tested for but it could not be demonstrated.

Method II. 0·001–0·01 per cent. *Solanum nodiflorum.* Bodies similar to those produced by method I were formed in the cells of the leaves in about two days.

Method III. 0·005 per cent. *Solanum nodiflorum.* Inclusion bodies were produced in a few leaves above the treated one.

Method IV. 0·01 per cent. *Solanum nodiflorum.* After repeated attempts the formation of rather indefinite bodies was induced in cells near the region of the puncture.

The results obtained with ammonium molybdate suggested the employment of a number of other substances. It was not possible to use molybdic acid as this is almost insoluble. An account follows of experiments with two other salts of molybdic acid and also a number of compounds containing the elements of the same group as molybdenum in the periodic system.

Sodium molybdate, potassium molybdate.

Method I. 0·05 per cent. *Solanum nodiflorum.* Inclusion bodies were formed in cells just as with ammonium molybdate.

Sodium tungstate.

Method I. 0·1 per cent. (pH$=6{\cdot}6$). *Solanum nodiflorum.* This reagent first stimulated and later caused some general coagulation of the cytoplasm.

Uranium acetate.

Method I. 0·1 per cent. *Solanum nodiflorum.* Slight stimulation was followed by general coagulation of the cell contents.

Selenious acid.

Method I. 0·1 per cent. Toxic.

0·01 per cent. *Solanum nodiflorum.* Plants became very stunted, and very small white leaves were formed as after treatment with the salts of molybdic acid. In the plant cells, slight stimulation was followed by coagulation. Numerous strands of cytoplasm passed across the vacuole of the cell.

Potassium dichromate.

Method I. 0·1 per cent. Toxic.

0·01 per cent. *Solanum nodiflorum.* Stimulation of cytoplasmic streaming was followed by an apparent increase in viscosity of the plasm.

Chromic acid.

Method I. 0·1–0·05 per cent. *Solanum nodiflorum, Nicotiana tabacum, Hyoscyamus niger.* There was a great increase in the rate of streaming of the cytoplasm. Small, spherical, hyaline bodies appeared in the cytoplasm. These minute bodies then disappeared and all the cytoplasm seemed to increase in viscosity.

Ammonia.

Method I. 0·1 per cent. *Solanum nodiflorum.* This reagent stimulated the cytoplasm to increased activity which was later followed by slight coagulation.

0·1 per cent. ammonium chloride produced similar effects.

ATTEMPTS TO INHIBIT THE DEVELOPMENT OF INCLUSION BODIES IN VIRUS-DISEASED PLANTS.

Methods.

The method of application of the reagents was essentially that of method I (p. 437), but a much larger number of plants was involved for each experiment. For each reagent used, two series of plants were set up, one series receiving the reagent only, the second series being also inoculated with the virus. Two series of controls were also set up, one receiving water only, the second receiving water but being inoculated with the virus at the same time as the experimental plants.

The time of inoculation with the disease was varied in the different experiments. Relative times of inoculation and treatment were suggested by the periods required for microscopic symptoms of the disease and for the cytological effects of the reagents to become apparent. Therefore some inoculations were made at the time the first dose of reagent was given; others were made during the course of the treatment.

Experiments with Solanaceae.

Solanum nodiflorum and *Nicotiana tabacum* were the host plants used in these experiments. Attempts were made to inhibit the development of inclusion bodies usually produced in these hosts by aucuba mosaic disease and by tobacco mosaic disease.

The strength of the reagents to be used was determined by trial. The following were selected:

Salicylic acid. 0·02 per cent. Each plant received ½ litre in three doses.

Potassium iodide. 0·02 per cent. Each plant received ½ litre in three doses.

Potassium bromide. 0·05 per cent. Each plant received 1 litre in three doses.

Ammonium thiocyanate. 0·05 per cent. Each plant received ½–1 litre in three to six doses.

Barbitone. 0·01 per cent. Each plant received 1 litre in six doses.

The results of these experiments were disappointing. Although after treatment the cytoplasm of the uninoculated plants appeared much less conspicuous, in all the inoculated plants inclusion bodies developed. The only noticeable effect was a retardation of symptoms in the plants treated with ammonium thiocyanate. It was thought that this ill success might be due to the acidity of the cell sap, as peptising agents often function best in alkaline media. Most plant juices lie in the acid range; this is true of all the Solanaceae used in these studies. It became necessary therefore to find a plant with an alkaline juice which would produce inclusion bodies on infection with a virus disease.

Experiments with Cucumis sativus.

B. N. Uppal, whilst working in this department, found the juice of the cucumber to be definitely alkaline. The most common virus disease of cucumber is the cucumber mosaic disease, but this produces no intracellular inclusions. It has been suggested that the development of inclusion bodies may be dependent on the *p*H of the plant tissue (15), and as the mosaic disease produces no inclusions in the cells of cucumber, it might be thought that their development is inhibited by the alkalinity of the sap. However, Hoggan failed to find inclusions in a number of other plants with acid juice which are susceptible to this disease (8). The writer has also failed to find inclusion bodies in *Solanum nodiflorum*, *Nicotiana tabacum*, *N. glutinosa*, *Hyoscyamus niger* and Zinnia infected with cucumber mosaic.

There are very few viruses to which cucumber is susceptible. Wingard (19) described a ringspot disease of tobacco as producing systemic infection in *Cucumis sativus*, and Woods (20) has demonstrated inclusion bodies in several *Nicotiana* species infected with this disease. Dr Kenneth Smith, who had obtained the virus from Dr Price, kindly supplied the author with it.

This tobacco ringspot was inoculated into *Nicotiana tabacum* var. White Burley, *Solanum nodiflorum* and *Cucumis sativus*. Inclusion bodies were produced in the cells of these plants. They were of the "amoeboid" form similar to the X-bodies produced in many hosts by tobacco mosaic virus. In White Burley tobacco, striate material was also produced, although Woods (20) did not describe this as occurring in Turkish or Havana seed-leaf varieties of tobacco. Striate material was produced also in *Solanum nodiflorum*, but it was not formed in *Cucumis sativus*. In the latter, only a very small proportion of the cells contained inclusion bodies. Plants were usually inoculated on the cotyledons when the first leaf was open. A violent chlorosis was produced on the second and third leaves. It was in the cells of these two leaves that inclusion bodies were occasionally found. Although the plants remained stunted they did to a certain extent grow out of the effects of the virus. Later leaves showed little chlorosis and enations developed on the lower surface. No intracellular inclusion bodies were ever found in these later leaves. It will be seen that cucumber infected with tobacco ringspot did not promise to be a very favourable subject for a study of the effects of peptising agents on virus-diseased material. However, in the absence of more favourable material, several experiments were made.

The reagents used were those described above, but it was usually found necessary to reduce the dose to one-half of that given to the Solanaceous hosts. In different experiments, inoculations were made on the day treatment commenced, and on one or two days after.

The *p*H of the juice of the second and third leaves of the control plants was estimated by the quinhydrone method: it was found to be 7·6.

Again no positive results were obtained. Inclusion bodies were found in at least one inoculated plant of each series. The bodies were found very infrequently, but owing to their comparative rarity in control plants, it was not possible to determine whether any of the treatments had brought about any diminution in the number of cells so affected.

DISCUSSION.

THE DIAGNOSTIC VALUE OF INTRACELLULAR INCLUSIONS.

The possible value of the cytological characteristics of diseased tissue in the diagnosis and classification of viruses has often been discussed. It is obviously not possible to identify a particular virus disease by these features alone, although each virus seems to be consistent in the type of inclusion it produces in a range of host plants. Both Hy. III and aucuba mosaic disease invariably cause the appearance in the cytoplasm of small particles which aggregate and fuse, the mature body, however, being slightly modified in form by different hosts. The bodies produced by these two diseases are indistinguishable morphologically, but they differ greatly from the amoeboid X-bodies of tobacco mosaic. The latter also appear always to be accompanied by striate material. Ringspot disease seems invariably to produce similar amoeboid bodies, whilst cucumber mosaic apparently causes no cytological irregularities. It would thus appear that the presence or absence of intracellular inclusions and their morphology when present should be of very considerable value in supplementing other diagnostic features.

THE NATURE OF INTRACELLULAR INCLUSIONS.

In a series of experiments such as those described in this paper, the possible variations in detailed conditions are almost infinite, but in each experiment the correct combination of conditions must be arrived at before the desired results can accrue. Available data on which to base experimental methods is particularly scanty, and consequently in this rather cursory study favourable results had of necessity to depend largely on the chance selection of the proper procedure. The plants responded to a certain extent to most of the coagulating agents. Almost without exception, these reagents induced an increased activity of the cell contents which flowed more freely whilst becoming more conspicuous. This effect is probably similar to that of drugs, which in small doses will stimulate, but it is also analogous to the action of certain viruses which, prior to the formation of inclusion bodies, stimulate the cytoplasm to increased activity. It is probable that more detailed investigations of many of these reagents would result in the production of further abnormalities. Throughout the experiments very little regard was had to either the *p*H or to the osmotic pressure of the solutions used. Although possibly of paramount importance, by method I these factors could not in any way be controlled, as the result of contact with the soil could not be estimated. Thus the negative results recorded here can be regarded as in no way conclusive.

With regard to the work on coagulants, the main object has been attained. It has been found possible, in the absence of the etiological agent of the disease, to parallel all the cytological phenomena induced by a virus. The salts of molybdic acid induced changes in the cell contents similar to those produced by aucuba mosaic and by Hy. III diseases. Soon after treatment the cytoplasm flowed more rapidly about the cell, then minute particles became evident and were carried passively about the cell by the cytoplasmic stream. These particles fused together until all were agglomerated into a single large body. The mode of formation of inclusion bodies in aucuba mosaic and in Hy. III diseases suggests them to be coagulation products of the cytoplasm, a view which is supported by the fact that similar changes can be wrought in the cytoplasm by physico-chemical experimentation.

Treatment with lactic acid occasionally causes a minute portion of the cytoplasm of a cell to become slightly differentiated from the rest in such a way that it could remain intact for some hours as it was carried about the cell and buffeted by the streaming plasm. These bodies are in appearance very like the X-bodies of tobacco mosaic disease, suggesting that the latter, although differing in appearance from the inclusions of aucuba mosaic disease, are of essentially similar origin, for both types of body can be artificially induced in healthy cells by similar treatments.

The substance of the inclusion bodies appears to consist of material precipitated or coagulated from the cell sap or cytoplasm due either to the action of the virus itself or to some substance or a change in physical conditions produced by unbalanced metabolism resultant on virus infection. Distinction has been drawn between the rounded inclusion bodies and the more crystalline structures such as the striate material of tobacco mosaic disease. These two types of inclusion may differ in chemical nature as do probably inclusions produced by different viruses or possibly those produced by the same virus in different hosts. But their origin would seem to be essentially similar. The fact that the striate material does in some cases fuse into a single mass lends further support to this view.

Of the results of the experiments designed to inhibit the development of inclusions little can be said, except to emphasise the difficulty of adequately controlling conditions within the cell. It is hoped that new methods of approach may bring more conclusive results.

SUMMARY.

The intracellular changes resultant on infection with aucuba mosaic and Hy. III diseases are described and are compared with the cytological effects of tobacco mosaic virus. With the two former viruses, inclusion bodies are formed by the aggregation and fusion of minute particles which appear in the cytoplasmic stream. With tobacco mosaic disease an amoeba-like body is produced and this persists for some weeks before suddenly disappearing again. It is accompanied by striate material all of which ultimately fuses into one large body.

Attempts have been made to parallel these conditions in healthy cells of Solanaceous plants by treatment with substances known to coagulate protoplasm. Almost all the reagents used induced stimulation of the cytoplasmic stream similar to the initial sign of virus infection. With salts of molybdic acid, all the cytological abnormalities due to aucuba mosaic or Hy. III disease have been imitated. Treatment with lactic acid induces the formation of amoeboid bodies like the X-bodies of tobacco mosaic, but these bodies persist for only a few hours.

Attempts have also been made to inhibit the formation of inclusion bodies induced by several different diseases in a number of hosts but no success was obtained.

The experiments support the view that the intracellular inclusions of plant virus diseases are essentially products of the host cell.

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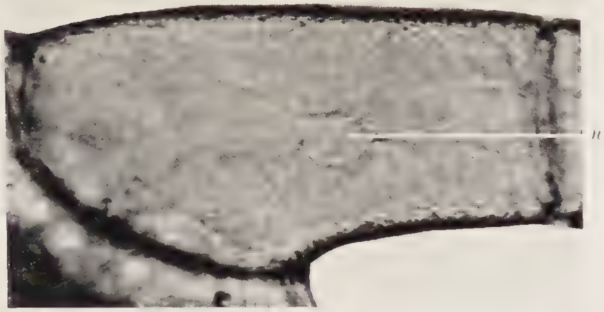

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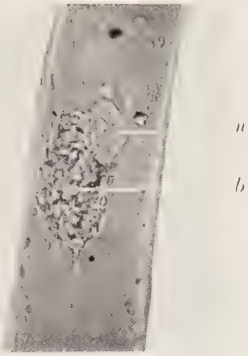

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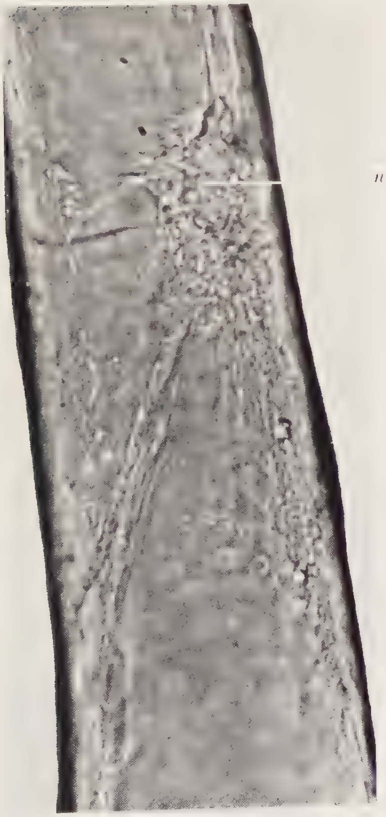

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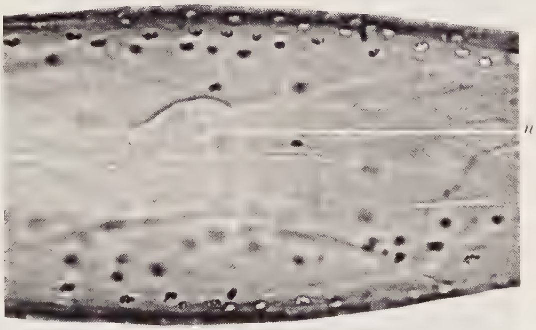

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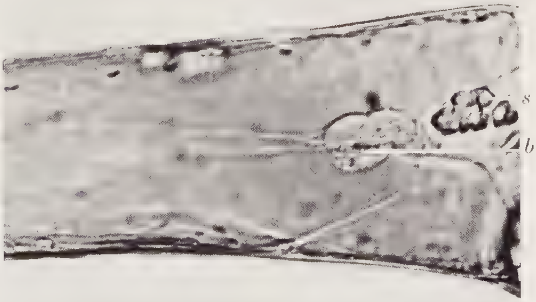
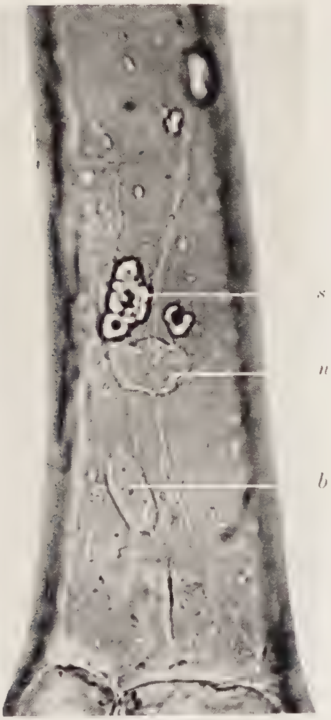


Fig. 5.

SHEFFIELD.—Experiments Bearing on the Nature of Intracellular Inclusions in Plant Virus Diseases (pp. 430–453).



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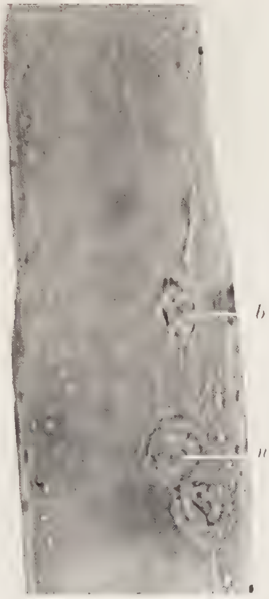

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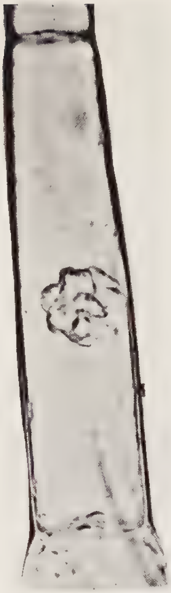

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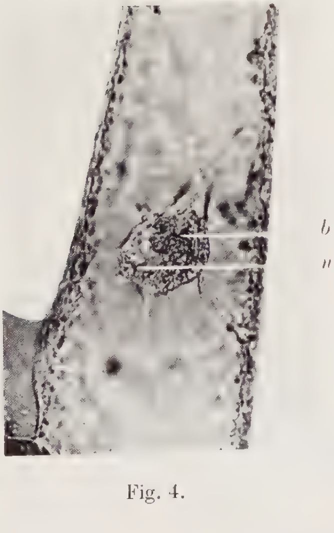

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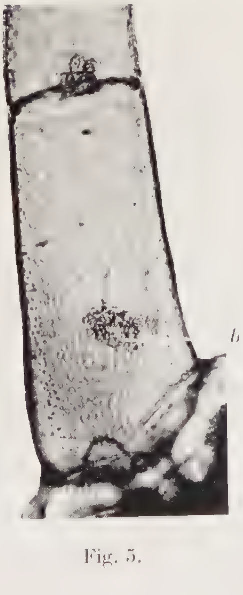

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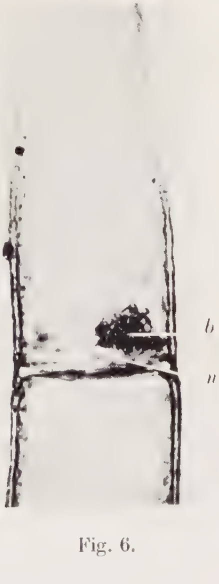
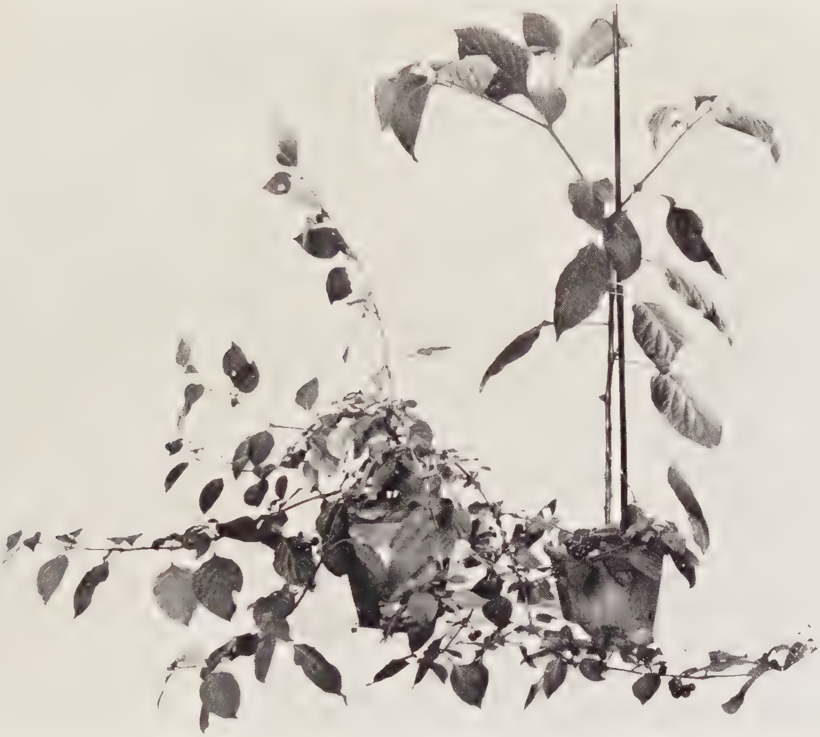


Fig. 6.

SHEFFIELD.—Experiments Bearing on the Nature of Intracellular Inclusions in Plant Virus Diseases (pp. 430–453).



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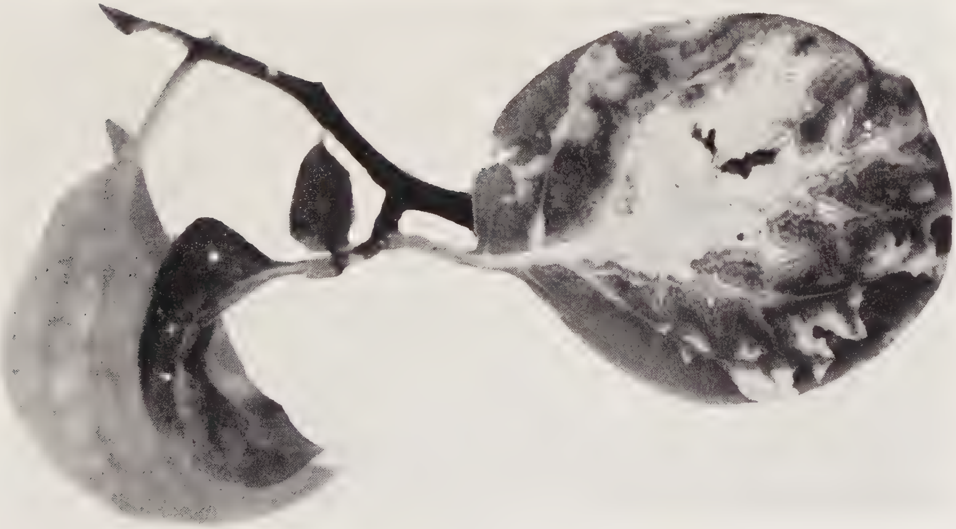

Fig. 2.

SHEFFIELD.—Experiments Bearing on the Nature of Intracellular Inclusions in Plant Virus Diseases (pp. 430–453).

EXPLANATION OF PLATES XXIII—XXV.

Some photomicrographs were taken with a Leitz Micca, others with a Leitz Makam camera. A Leitz 6 L objective was used in combination with a Leitz periplanat eyepiece either ×8, ×10, or ×12. Photographs taken with the Micca camera were enlarged three times for reproduction; those taken with the Makam have been reproduced unaltered.

All photomicrographs were taken from unstained living cells.

The following symbols are used to label the figures: *b* = intracellular inclusion body; *n* = cell nucleus; *s* = striate material.

PLATE XXIII.

Fig. 1. *Solanum nodiflorum.* Basal cell from hair of normal healthy plant. The nucleus is suspended in the centre of the cell by a few delicate cytoplasmic strands. ×450.

Fig. 2. *Nicotiana tabacum.* Hair cell a few days after infection with tobacco mosaic disease. The cytoplasm is much more conspicuous than in the normal cell and appears to have increased in bulk. ×525.

Fig. 3. *Nicotiana tabacum.* Inclusion body produced in cell of trichome as a result of infection with Hy. III disease. ×450.

Fig. 4. *Solanum nodiflorum.* Cell from hair soon after treatment with chloroform. The cytoplasm has become more conspicuous and has increased in bulk. ×450.

Fig. 5. *Nicotiana tabacum* with ring spot disease. An amoeboid inclusion body and striate material have formed in the hair cell. ×450.

PLATE XXIV.

Fig. 1. *Nicotiana tabacum* with tobacco mosaic disease. An amoeboid X-body and striate material are present in the hair cell. ×500.

Fig. 2. *Solanum nodiflorum* after treatment with lactic acid. An inclusion, morphologically similar to the X-bodies of Plate XXIII, fig. 5 and Plate XXIV, fig. 1, is present in this hair cell. ×450.

Fig. 3. *Solanum nodiflorum.* Six weeks after infection with tobacco mosaic disease, the amoeboid X-body has disappeared and the striate material has formed a single large mass. ×400.

Fig. 4. *Solanum nodiflorum.* Inclusion body produced in cell of trichome on infection with aucuba mosaic disease. ×450.

Figs. 5 and 6. *Solanum nodiflorum.* Inclusion bodies morphologically similar to those of Plate XXIII, fig. 3, and Plate XXIV, fig. 4, are produced in cells as a result of treatment with ammonium molybdate. ×400.

PLATE XXV.

Fig. 1. *Solanum nodiflorum.* On the right, a healthy plant two months old showing the normal erect habit. On the left, a plant five months after treatment with ammonium molybdate. This plant is much branched, it has a trailing habit and small leaves. A few of the older leaves show the initial typical poison mottle.

Fig. 2. *Solanum nodiflorum.* Branch of plant six months after treatment with ammonium molybdate. One leaf bears a mottle similar to that produced by a virus. All other leaves of the branch are a uniform yellowish green colour. ×1½.

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THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS

PART IX. THE AEROBIC DECOMPOSITION OF HEMICELLULOSES

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I. INTRODUCTION.

THE decomposition of hemicelluloses by micro-organisms has, until quite recently, received little attention, even though representatives of this rather indefinite group of polysaccharides are present to a greater or lesser extent in practically all types of plant materials. Not infrequently, next to cellulose, do they form the largest carbohydrate constituent, and accordingly their availability may markedly influence the rate of decomposition of the tissue as a whole. Their importance in industrial fermentations involving natural materials seems often to have been overlooked. Waksman and Diehm (7) have recently reviewed in a detailed manner the literature dealing with the distribution and degradation of the hemicelluloses under natural conditions, and no useful purpose would be served by repeating these citations.

Very few investigations of the decomposition of isolated hemicelluloses in a pure state have been recorded, partly because their preparation in a satisfactory condition presents some difficulties, and partly because accurate methods of estimation and, therefore, of following the decomposition are lacking. Though there are some cases in which there is only one sugar unit involved, more generally two and sometimes three sugars are present. Both hexoses and pentoses are commonly present together with a hexose uronic acid. These form the polyuronides which are the common cell wall incrusting materials in most plant materials and woods. Associated intimately with the cellulose, and isolated with it, is another form of hemicellulose, free or almost free of uronic acid units, and in general of only one sugar unit. These have been termed cellulosans (1). Their rôle is different to that of the encrusting polyuronides, for they are an integral part of the cellulose framework of the plant. In most materials the polyuronides are quantitatively more important than the cellulosans, which are presumably distributed evenly throughout the cellulose and therefore cannot be utilised to any extent unless the cellulose also is attacked. Preparations made by the alkaline extraction of materials, such as straw, contain both polyuronides and cellulosans, and there is as yet no method of effecting a separation. Since the polyuronides are the more soluble, they usually predominate. The linkage in both classes of hemicellulose seems to be of a similar nature and, from a chemical point of view, relatively strong and fairly resistant to mild acid hydrolysis. Hemicelluloses are distinctly more stable towards acids than starch, glycogen or pectin, but much less stable than true cellulose. The structural heterogeneity of individuals of this group raises interesting possibilities of the unequal assimilation of constituent units. That they are readily available to micro-organisms is clear from the work of Rege (4), the earlier work of Waksman (both of which workers only estimated the furfural yielding groups, expressing these rather unfortunately as pentosans), and from that described in earlier papers of this series in which an attempt was made to differentiate between the various furfural-yielding constituents and groups of the hemicelluloses. Not only are they extensively attacked, but their removal is accomplished soon after decomposition has set in, and before the main bulk of the cellulose is lost. In a normal aerobic rot with a mixed natural flora the hemicelluloses seem to provide abundant readily available material for the development of a large and active flora, thus paving the way for the attack on the cellulose, which in materials deficient in hemicelluloses or from which this fraction has been removed, is notably slower. Observations on mixed flora decompositions and indirect evidence from nitrogen immobilisation figures suggest that fungi develop first and probably are responsible for much of the observed loss of hemicelluloses. Accordingly in the work to be described many fungi were tested as to their ability to utilise a natural hemicellulose preparation, and a search made for aerobic bacteria which would also do so.

Whilst this work was in progress Waksman and Diehm (8, 9) published the results of a study on somewhat similar lines. They employed as substrates preparations of so-called "pentosan" from Dulse (a seaweed), mannan, galactan, and xylan, only the last of which could reasonably be regarded as pure. It has yet to be shown that the pentosan from Dulse, or the galactan from Irish Moss, are similar in composition to the hemicelluloses of the higher plants, and since the total hemicellulose in the galactan amounted to only a little over 50 per cent., and in the mannan and pentosan to less than 75 per cent., there is no certainty that any hemicellulose was really utilised when only a partial fermentation occurred. A number of organisms isolated from an enrichment culture in the "pentosan" were unable to utilise xylan. Similarly, since the preparations with one exception were not nitrogen free, the figures for the amount of carbohydrate decomposed per unit of nitrogen assimilated are not absolute values, for all, or a part, of the organic nitrogen of the preparation may also have been utilised. In spite of these objections to the quantitative aspects of their survey, these workers have shown that there is in the soil an abundant flora of all types capable of utilising hemicelluloses to some extent.

II. Experimental.

The source of the crude hemicellulose preparation used in this work was oat straw, from which it was prepared by the usual means, namely extraction with warm 4 per cent. NaOH after pretreatment with alcoholic soda to remove a portion of the lignin. The extract was acidified with acetic acid after filtration through glass-wool and sufficient alcohol added for complete precipitation. The precipitate was purified by solution in alkali and reprecipitation. A portion was also fractionated into preparations A and B, the former by the addition of an excess of glacial acetic acid to the crude solution, and the latter by the addition of alcohol or acetone to the acetic acid filtrate. All preparations were low in ash and nitrogen free. The crude preparation used in most of the work to be described gave 2·04 per cent. CO_2 from the uronic acid present, and 34·0 per cent. furfural from the pentose and uronic groups. This corresponds approximately with 8·2 per cent. uronic acid and 50·8 per cent. pentose, expressed as xylan, the remaining 41 per cent. presumably being hexosan.

(i) *Hemicellulose-decomposing fungi.*

That some fungi are capable of utilising the hemicelluloses of natural materials has been known for some time, and shown directly by the work of Waksman (6) and Norman (3) in pure culture. They have further been suspected of playing a large part in the removal of this group in rots brought about by a mixed natural flora of bacteria and fungi together. There is under ordinary conditions a rapid removal of the hemicelluloses in the early stages of fermentation, and coincident with this there is observed to be a very considerable development of fungal tissue. In general, fungi seem to be less specialised than most bacteria in their nutritional requirements. Materials such as straw rotted by a single fungus give on analysis figures very similar to those obtained on partial fermentation with a mixed flora, and all groups of plant constituents are attacked. Therefore it seemed likely that many fungi can utilise hemicelluloses, and that the isolation and identification of well-known forms from enrichment cultures would not be of much value. Accordingly it was decided to make a qualitative test of the utilisation of hemicelluloses by a large group of common fungi. Of all groups in nature the Aspergilli and Penicillia seem to be the commonest and most ubiquitous. Nearly seventy organisms were tested, many of them being of one or the other of these groups.[1] They were transferred from slopes on glucose-peptone to Petri plates with medium of the following composition: agar 15 gm., hemicellulose 10 gm., peptone 5 gm., $(NH_4)_2HPO_4$ 1 gm., KCl 0·5 gm., $MgSO_4$ 0·5 gm., Fe salt trace, per litre, $pH = 7·0$. The hemicellulose goes into solution on autoclaving and the medium on cooling is opalescent and creamy brown in colour. No hydrolysis takes place under this treatment and the medium is non-reducing. The inoculation was a small one on the centre of the plate and done in quadruplicate. At the same time four other plates were set up with a medium similar in composition, except that glucose was substituted for the hemicellulose, and two plates with a medium containing the other constituents but no carbohydrate. This was to distinguish between growth at the expense of carbohydrate and growth on the peptone, the concentration of which was rather high. At the end of two days and a week the growth was compared, the colony diameter measured and general appearance assessed. In some cases there was a marked ring or cleared zone round the colony on the hemicellulose medium indicating the production of an exo-enzyme. On the medium containing peptone and inorganic salts alone growth was in all cases meagre, scanty, or nil. Table I gives the comparative development on glucose and hemicellulose medium at the end of 2 and 7 days.

---

[1] I am indebted to Dr H. C. Greene for these cultures.

The most striking feature of the results obtained was the fact that every organism which was tested grew reasonably well on the hemicellulose medium, the majority at least as well as on glucose, and quite a number better than on glucose. Inasmuch as the cultures tested were selected entirely at random this was somewhat surprising and indicates that the ability to utilise hemicelluloses of this type is widespread amongst fungi. The cleared zone round the colony was a prominent feature on many plates. In general this appeared quite soon after inoculation and sometimes became less obvious as the colony enlarged. Those cases marked † in Table I still exhibited a wide cleared area at the end of 7 days, when in most cases the colony had reached almost its maximum growth. This property of the production of an exo-enzyme might possibly be of value in distinguishing between certain closely allied Penicillia or Aspergilli.

(ii) *Hemicellulose-decomposing bacteria.*

The method of approach in the case of bacteria was somewhat different and, instead of testing known organisms for their ability to decompose hemicelluloses, isolations were made from various natural sources.

The following media were employed:

Medium HA, per litre:—20 gm. purified hemicellulose (oat straw), 5 gm. peptone, 1 gm. KH_2PO_4, 0·5 gm. KCl, 0·5 gm. $MgSO_4$, trace $FeSO_4$, 15 gm. agar, $pH=7{\cdot}6$.

Medium HAL. The above without the addition of agar.

Medium HB, per litre:—10 gm. purified hemicellulose, 1·0 gm. NH_4NO_3, 1·0 gm. K_2HPO_4, 0·5 gm. $MgSO_4$, 0·5 gm. NaCl, 0·5 gm. peptone, trace $FeSO_4$, 15 gm. agar, $pH=7{\cdot}0$.

Medium HBL. The above without the addition of agar.

From soil enrichment cultures, decomposing straw, and manure a considerable number of aerobic organisms were isolated. Inasmuch as non-hemicellulose decomposing organisms will develop on the peptone in medium HA, the following criteria are necessary and were applied. To be regarded as a hemicellulose decomposer an organism must either

Table I.

Growth of fungi on hemicellulose medium.

Organism				2 days	7 days
Aspergilli					
Aspergillus sp.	4235	X 4	Thom	$H=G$	$H>G^*$
A. fumigatus				$G>H$	$H>G^*$
A. ochraceus	4700 *a*			$G>H^*$	$H=G$
A. oryzae	No. 965			$G>H$	$H>G\dagger$
A. parasiticus	3509		Thom	$H=G^*$	$G>H$
A. schiemanni	3534 C			$G>H^*$	$G>H$
A. spadix	4707	757		$H=G^*$	$H=G$
A. sydowi	5128	5114	Thom	$H=G^*$	$H=G$
A. tamarii	4735			$H=G$	$H=G$
A. terricola var. *americana*	4838			$H=G$	$G>H$
A. ustus	3556		Thom	$H=G$	$G>H^*$
A. wentii	44173			$H=G$	$H=G$
Aspergillus sp.	Taka.	N. 292	Thom	$H=G^*$	$G>H^*$
Aspergillus sp. 1(? *niger*)				$H=G^*$	$H>G$
Cephalothecium sp.				$H=G^*$	$G>H^*$
Clonostachys araucaria	5128	5113	Thom	$H=G^*$	$H=G$
Coprinus radians				$G>H$	$G>H^*$
Cunninghamella sp.				$G>H$	$H=G$
Fomes annosus				$H=G$	$H=G$
Gliocladia					
G. atrum	4894	18	Thom	$G>H$	$H=G$
G. deliquescens	5128	5101, 1	Thom	$G>H$	$H=G$
G. fimbriatum	5128	5101, 3	Thom	$G>H$	$H=G$
G. roseum	5128	5101, 2	Thom	$H=G$	$H>G^*$
G. vermoeseniu	5090	4 *a*	Thom	$H=G$	$G>H^*$
Lenzites lepidus				—	$H=G$
Monascus sp.				$H=G$	$H=G$
Monilia sitophila				$G>H$	$H=G$
Mucor sp.				$G>H$	$G>H$
Paecilomyces varioti (syn. *Penicillium divaricatum*)				$G>H$	$H=G$
Penicillia					
Penicillium anisopliae (*Metarrhizium*)	1367		Thom	$H=G^*$	$H=G$
P. atramentosum	4733	3	Thom	G —	$G>H$
P. aurantio-brunneum	4733	5	Thom	$G>H$	$H=G\dagger$
P. avellaneum	4401		Thom	$H=G$	$H=G$
P. caseicolum Hastings	1, 17, A			$H=G$	$H=G$
P. chloro-leucon	4733	30	Thom	$H=G$	$H=G\dagger$
P. chrysogenum	4733	33	Thom	$H=G$	$H=G$
P. chrzaszczi	5101	7	Thom	— —	$H=G$
P. citreo-viride	4733	38	Thom	$H=G$	$G>H$
P. citrinum	4482		Thom	$H=G^*$	$H=G\dagger$
P. commune	23		Thom	$H=G$	$G>H$
P. cyaneo-fulvum	4733	47	Thom	$H=G$	$H=G\dagger$
P. cyaneum	4640	422	Thom	$H=G^*$	$H=G^*$
P. digitatum				$G>H$	$G>H\dagger$
P. elegans	5129 B		Thom	$H=G$	$H>G$
P. expansum 1, H. Greene				— —	$H=G$
P. granulatum	4641		Thom	$G>H$	$G>H$
P. granulatum 1, H. Greene				$H=G$	$H=G$
P. Hagemi	5010	8	Thom	$H=G$	$H>G^*$
P. islandicum	4785	7	Thom	$H=G^*$	$G>H^*$
P. janthinellum	4141		Thom	$H=G$	$\ddagger G>H\dagger$
P. Jenseni	5010	10	Thom	$H=G^*$	$G>H\dagger$
P. Johannioli	5010	11	Thom	G —	$H=G^*$
P. lilacinum	4855		Thom	$H=G$	$H=G$
P. luteum	1124			$G>H$	$G>H^*$

Table I (*cont.*).

Organism				2 days	7 days
P. matris-meae	5010	14	Thom	$G>H$	$H>G$
P. oxalicum	Soil		Thom	$G>H$	$H>G$*
P. purpurogenum	1128			$G>H$	$H=G$†
P. Racidborskii	5010	19	Thom	G —	$H=G$
P. roqueforti	4733	102	Thom	$G>H$	$H=G$
P. sanguineum	4917	7	Thom	$G>H$	$H>G$*
P. solitum	2546		Thom	$H=G$	$H=G$
P. spinulosum	45		Thom	$G>H$	$G>H$
P. terrestre	5034	8	Thom	$H=G$	$G>H$
P. verrucosum	4733	125	Thom	$H=G$	$H=G$
Rhizopus nigricans				$G>H$	$G>H$
Sclerotium rolfsii				$H>G$	$H>G$†
Syncephalastrum sp.				$H=G$	$H>G$

KEY: $H>G$, organism developed better on hemicellulose than on glucose.
$H=G$, organism developed as well on hemicellulose as on glucose.
$G>H$, organism developed better on glucose than on hemicellulose.
* Cleared zone round colony on hemicellulose medium.
† Very marked clearing.
‡ Sectoring of colony.

grow on medium HB, produce a clearing of the semiopaqueness of media HBL and HAL, or show a cleared zone round the colony on medium HA. Not every organism which clears medium HAL will produce such a zone on the agar medium HA. In point of fact every organism described grew to a greater or lesser extent on the liquid medium HBL, though some developed more actively when supplied with nitrogen in an organic form. Isolations were made at 30 and 65° C. The following seventeen mesophilic organisms, five of which are closely related species, and three thermophilic organisms, were isolated and characterised.

Morphological characteristics.

(*a*) *Mesophilic organisms.*

No. 1.

Source	Soil.
Medium	Clearing HAL.
Morphology	Rods 2·0–2·5 × 0·5–0·7, occurring often in chains and in bundles. Many spores at 42 hours. No swelling. Spore size 2·0–2·5 × 1·0–1·2, thickened at end.
Gram stain	Strongly positive.
Gelatin stab	Liquefaction.
Agar plates	Circular, smooth, flat, edge lobate, very coarsely granular.
Agar plants	Spreading, flat, dull, smooth, translucent, no colour, consistency butyrous, medium unchanged. Visible growth 4 hours.
Agar shake tubes	Aerobic.
Broth	Surface growth and pellicle 12 hours, heavy sediment 2 days.
Litmus milk	Reduction of litmus 5 days, peptonisation by 12 days, no coagulation.
Potato	White, spreading.
Catalase	Positive, very strong.

No. 2.

Source	Soil.
Medium	Clearing HAL.
Morphology	Rods 1·6–2·0 × 0·5–0·7, arranged in bundles. Spores few at 60 hours. Spore size 1·0 × 2·5. No swelling.
Gram stain	Negative.
Gelatin stab	Liquefaction.
Agar plates	Circular, smooth, flat, edge lobate, coarsely granular.
Agar slants	Spreading, flat, dull, smooth, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake tubes	Aerobic.
Broth	Surface growth and light pellicle, 12 hours. some sediment 2 days.
Litmus milk	Slight reduction of litmus by 60 hours, peptonisation by 12 days, no coagulation.
Potato	Thick, white, gummy.
Catalase	Positive, strong.

No. 3.

Source	Soil.
Medium	Clearing HAL.
Morphology	Rods 1·6–2·0 × 0·8–1·0, occurring in bundles. Spores at 60 hours. No swelling. Spore size 1·0 × 2·0, end thickened.
Gram stain	Strongly positive.
Gelatin stab	Liquefaction.
Agar plates	Circular, smooth, flat, edge undulate, finely granular.
Agar plants	Spreading, flat, dull, smooth, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake	Aerobic.
Broth	Surface growth, some flocculum 12 hours, pellicle, 2 days.
Litmus milk	Coagulation 36 hours, complete peptonisation 9 days.
Potato	Thick, white, gummy.
Catalase	Positive, very strong.

Nos. 4, 6, 12, 14, 15.

Source	4 and 6 soil; 12, 14, and 15 rotting straw.
Medium	HBL.
Morphology	Short rods 1·5 × 1·0 and smaller. No spores. Rods become smaller and coccoid with time.
Gram stain	Negative.
Gelatin stab	No liquefaction.
Agar plates	Circular, smooth, raised (concave). Edge entire to slightly undulate becoming lobed, amorphous, star forms in centre of colony.
Agar slants	Growth abundant, echinulate, raised, glistening opalescent, no colour, consistency viscid, medium unchanged.
Agar shake	Aerobic, facultative, gas evolution.
Broth	Turbid, gas evolution and pellicle at 12 hours, flocculant ring 2 days.
Litmus milk	Slight acidity 12 hours, acid and gas 36 hours, reduction of litmus and coagulation 60 hours, gas production but no peptonisation.
Potato	4 and 14, spreading. 6, thick whitish, heavy. 12, restricted, gummy. 15, whitish, wrinkled.
Catalase	4, positive, weak. 12, positive, very strong. 6, 14, 15, positive, strong.

No. 5.

Source	Soil.
Medium	Clearing HAL.
Morphology	Rods 1·5–2·5 × 0·5–0·8 in definite chains. Spore formation without change of shape at 24 hours. Spore size 1·5–2·0 × 1·0–1·2.
Gram stain	Negative.
Gelatin stab	Liquefaction.

Agar plates	Circular, smooth, raised margin, edge vague, granular.
Agar slants	Spreading, flat, dull, smooth, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake	Aerobic.
Broth	Turbid with pellicle, 12 hours, some sediment, 2 days.
Litmus milk	Reduction of litmus and coagulation 36 hours, complete peptonisation, 9 days.
Potato	Yellowish, moist.
Catalase	Positive, weak.

No. 7.

Source	Soil.
Medium	Clearing HAL.
Morphology	Rods 1·5–2·0 × 0·5–0·8 in bundles. Many show granulations. Spores at 42 hours. Spore size 2·0–2·5 × 1·0. Thickened at ends.
Gram stain	Positive.
Gelatin stab	Liquefaction.
Agar plates	Spreading, smooth, flat, deep lobes, granular.
Agar slants	Spreading, flat, dull, smooth, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake	Aerobic.
Broth	Pellicle 12 hours, some sediment 2 days.
Litmus milk	Reduction of litmus, coagulation and slow peptonisation.
Potato	White, heavy.
Catalase	Positive, strong.

No. 8.

Source	Manure.
Medium	Clear zone on HA.
Morphology	Rods 2·5 × 0·5–0·7, packed in bundles. Occasional pleomorphic forms up to 15 long. Spores rod-shaped at 84 hours. Spore size 1·0–2·0 × 0·8.
Gram stain	Negative, or slightly positive.
Gelatin stab	Slight liquefaction.
Agar plates	Spreading, slightly rough, edge irregular and thickened, faintly granular.
Agar slants	Growth moderate, echinulate, flat, dullish, contoured, translucent, no colour, consistency soft, medium unchanged.
Agar shake	Aerobic.
Broth	Turbidity at surface, 12 hours, general turbidity 2 days.
Litmus milk	No change to 6 days, some coagulation, and slight peptonisation 12 days.
Potato	Whitish, wrinkled.
Catalase	Positive, strong.

No. 9.

Source	Manure.
Medium	Clear zone on HB.
Morphology	Rods 2·0–2·5 × 0·5–0·8 showing clearly defined granulations. Spores at 60 hours, thickened at ends. Spore size 1·8–2·0 × 1·0–1·2.
Gram stain	Negative.
Gelatin stab	Liquefaction.
Agar plates	Spreading, smooth, flat, edge deeply lobed—irregular, coarsely granular.
Agar slants	Spreading, flat, dull, smooth, translucent, no colour, consistency dry, medium unchanged.
Agar shake	Aerobic.
Broth	Surface growth and pellicle, 12 hours.
Litmus milk	Coagulation by 36 hours, strong peptonisation, complete by 9 days.
Potato	White, very wrinkled.
Catalase	Positive, weak.

No. 10.

Source	Manure.
Medium	Clearing HAL.
Morphology	Rods 2·0–2·5 × 0·5–0·8, and occasionally longer. Spores at 84 hours, thick walled. Spore size 1·6–1·8 × 0·8–1·0.
Gram stain	Positive?

Gelatin stab	Liquefaction.
Agar plates	Spreading, smooth, flat, edge deeply lobed, very coarsely granular.
Agar slants	Spreading and arborescent, flat, dull, smooth, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake	Aerobic.
Broth	Surface growth and pellicle with some sediment, 12 hours, sediment 2 days.
Litmus milk	Reduction of litmus, coagulation 5 days, peptonisation steady but slow.
Potato	White, very wrinkled.
Catalase	Positive, very strong.

No. 11.

Source	Manure.
Medium	Clearing HAL.
Morphology	Rods 2·0–2·5 × 0·5–0·8, grouped in bundles. Spores at 42 hours without swelling. Spore size 1·6 × 1·0.
Gram stain	Negative.
Gelatin stab	Liquefaction.
Agar plates	Spreading, smooth, flat, edge deeply lobed and arborescent, very coarsely granular.
Agar slants	Spreading, flat, dull, thin, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake	Aerobic.
Broth	Surface growth and pellicle 12 hours; sediment 2 days.
Litmus milk	Reduction of litmus, and slow peptonisation from 6 days.
Potato	Slight growth.
Catalase	Positive, weak.

No. 13.

Source	Manure.
Medium	Clearing HBL.
Morphology	Long rods 3·0–4·5 × 0·5–1·0. Some pleomorphic forms up to 8. Spores at 42 hours, without swelling. Spore size 1·6–1·8 × 1·0.
Gram stain	Positive.
Gelatin stab	Liquefaction.
Agar plates	Irregular, smooth, flat, edge lobate, coarsely granular, or filamentous.
Agar slants	Spreading, flat, dull, thin, translucent, no colour, consistency butyrous to dry, medium unchanged.
Agar shake	Aerobic.
Broth	Faint turbidity 12 hours, pellicle, 2 days.
Litmus milk	Reduction of litmus and coagulation in 60 hours, slow peptonisation.
Potato	Whitish, wrinkled.
Catalase	Positive, weak.

No. 16.

Source	Manure.
Medium	Clearing HAL.
Morphology	Rods 2·0–2·5 × 0·5–0·8. Marked granulations. Spores at 42 hours, central without swelling. Spore size 1·5–1·8 × 0·6–0·8.
Gram stain	Negative. Few rods positive.
Gelatin stab	Liquefaction.
Agar plates	Circular, smooth, flat, edge lobate.
Agar slants	Spreading and arborescent, flat, glistening, smooth, translucent, no colour, consistency butyrous, medium unchanged.
Agar shake	Aerobic.
Broth	Surface growth and pellicle 12 hours, sediment 2 days.
Litmus milk	Reduction of litmus and coagulation in 60 hours, steady peptonisation.
Potato	White, very heavy growth.
Catalase	Positive, strong.

No. 17.

Source	Manure.
Medium	Clearing HAL.
Morphology	Rods 3·0–3·5 × 1·0. Granulation. No spores.

Gram stain	Negative.
Gelatin stab	Liquefaction.
Agar plate	Circular, smooth, flat, edge undulate, strongly granular, filamentous.
Agar slants	Growth moderate, separate groups, flat, glistening, smooth, translucent, no colour, consistency viscid, medium unchanged.
Agar shake	Aerobic.
Broth	Some turbidity 12 hours, turbidity 2 days, no pellicle or sediment.
Litmus milk	Coagulation in 36 hours, slight acid 60 hours, reduction of litmus and strong peptonisation.
Potato	White, very heavy growth.
Catalase	Positive, very strong.

(*b*) *Thermophilic organisms.*

X.

Source	Soil.
Medium	Clearing HA and HB.
Morphology	Long rods, 5·0–9·0 × 1·0, often with terminal spores swelling to club shape or even plectridium form. Size variable on liquid media, more constant on agar. Median band visible occasionally. Spore size 1·6 × 1·0 approximately.
Gram stain	Negative.
Gelatin	(Frazier method—tannic acid.) Liquefaction.
Agar plate	Spreading, thin, gummy.
Agar shake	Aerobic.
Broth	Turbid, no pellicle.
Litmus milk	Coagulation and some peptonisation.
Potato	Gummy growth.
Catalase	Negative, or very faint.
Starch agar	Good growth, marked clear zone round colonies.

Y.

Source	Manure.
Medium	Clearing HA.
Morphology	Long rods, 16·0–12·0 × 1·0. Variable in length, no swelling or visible spores, marked granulation, cells sometimes convoluted. Very pleomorphic in liquid culture.
Gram stain	Negative.
Gelatin	(Frazier method—tannic acid.) Liquefaction.
Agar plate	Spreading, thin, gummy.
Agar shake	Aerobic.
Broth	Turbid, no pellicle.
Litmus milk	Reduction of litmus. Coagulation and peptonisation 3 days, heavy peptonisation by 9 days.
Potato	Gummy growth.
Catalase	Negative.
Starch growth	Good growth, marked clear zone round colonies.

Z.

Source	Manure.
Medium	Clearing HA. and HB., preferring the latter.
Morphology	Very long rods resembling actinomycetes in liquid culture. Some shorter rods 5·0 × 1·0 and less. More constant on agar. No visible spores. Some granulation.
Gram stain	Positive.
Gelatin	(Frazier method.) No liquefaction.
Agar plate	Spreading, thin, gummy.
Agar shake	Aerobic.
Broth	Turbid, no pellicle.
Litmus milk	Coagulation and marked peptonisation 7 days.
Potato	Gummy growth.
Catalase	Negative.
Starch agar	Good growth, marked clear zone round colonies.

Biochemical reactions.

(*a*) *Utilisation of nitrogen.*

Medium: glucose 10 gm., K_2HPO_4 0·5 gm., NaCl 0·5 gm., $MgSO_4$ 0·2 gm. per litre, $pH = 7·0$.

1 gm. each per litre of KNO_3, KNO_2, ammonium phosphate, urea, and peptone added, the nitrite being sterilised separately and added to the tubes after sterilisation.

Brom-chlor-phenol-blue (range *p*H 3·0–4·8) was also added to the peptone and ammonium phosphate series in the case of the mesophilic organisms.

	Nitrate*		Nitrite	Ammonium phosphate		Urea	Peptone	
Organism	Growth	Reduction to nitrite	Growth	Growth	*p*H 1 day	Growth	Growth	*p*H 1 day
1	–	–	–	+	No change	+	+	No change
2	+	+	–	+	,,	+	+	,,
3	+	Trace	–	+	,,	+	+	,,
4, 6, 12, 14 and 15	+	+ +	–	+	4·0 + gas	+	+	3·5–4·0 + gas
5	+	+	–	+	No change	+	+	No change
7	–	–	–	+	,,	–	+	,,
8	–	–	–	–	,,	–	+	,,
9	+	–	–	+	,,	+	+	,,
10	+	Trace	–	+	,,	+	+	,,
11	+	Trace	–	+	,,	+	+	,,
13	+	–	–	+	,,	+	+	,,
16	–	–	–	+	,,	+	+	,,
17	–	–	–	–	,,	+	+	,,
X	+	+	–	–		–	+	
Y	–	–	–	–		–	+	
Z	+	+	–	+		–	+	

* When tested for nitrate reduction on the standard medium used for that test (glucose 2·0 gm., beef extract 2·5 gm., peptone 1·0 gm., KH_2PO_4 1·0 gm., KNO_3 1·0 gm. per litre) all organisms developed well and the pink colour indicative of nitrite, on addition of α-naphthylamine in acetic acid and sulphanilic acid, was given on plates by all except 7 and 8 and in broth by all except 7, 8, X, Y and Z. Some organisms which are unable to utilise and reduce nitrate when supplied with that alone, can seemingly do so when supplied as well with an organic source of nitrogen.

(*b*) *Indole production.*

No organism gave a positive test for indole when growing on a medium consisting of 1 per cent. peptone and 0·5 per cent. NaCl, either at 1 day or after 14 days' incubation.

(*c*) H_2S *production* (*Kligler's lead acetate method*).

Negative in all cases at 1 day and 1 week.

(*d*) *Reduction of methylene blue.*

At 1 day, marked reduction by 3 and 9, slight by 2, and at 4 days complete reduction by 2, 3 and 9.

(*e*) *Utilisation of carbohydrates.*

(i) *Gas production from glucose-peptone broth* (1 *per cent. glucose*). Considerable gas was given by the facultative group Nos. 4, 6, 12, 14 and 15, and only small quantities by any other organism.

(ii) *Acid production from glucose peptone broth* (1 *per cent. glucose*). Five c.c. of the broth used for the gas production test above were titrated with $N/10$ alkali after fermentation. The facultative group, 4, 6, 12, 14 and 15, gave a titration of about 0·7–0·8 c.c. $N/10$ alkali, while no other organism produced more than a trace of acid.

(iii) *Availability of sugars and polysaccharides.* The organisms were inoculated into a set of tubes in duplicate, containing a liquid medium of 1 per cent. carbohydrate together with 0·1 per cent. peptone and the usual mineral salts. The growth was observed, and the acid produced titrated with $N/10$ alkali at the end of 2 weeks. Every organism grew on every carbohydrate tested, but in few cases was there more than a trace of acid produced. In many cases there was either a sediment, or pellicle in addition to turbidity of the medium. No useful purpose would be served by giving the titrations in detail. The facultative group Nos. 4, 6, 12, 14 and 16 gave small quantities of acid from a number of sugars (in quantity up to the equivalent of 1·0 c.c. $N/10$ alkali per 5 c.c. culture solution). Most of the organisms showed some acidity on starch and salicin, and a few on glucose and sucrose. The carbohydrates tested were: arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, melizitose, α-methylglucoside, pectin, raffinose, rhamnose, salicin, starch, sucrose, and xylose.

In view of the ability of these organisms to utilise to some extent such a wide range of carbohydrates, it was hoped to effect some differentiation by presenting them with certain mono- and disaccharide acids of known linkage, and some less available polysaccharides. The following acids were employed in the form of the calcium salt:

(i) Gluconic acid,
(ii) Saccharic acid,
(iii) Maltobionic acid (4α-glucosido-gluconic acid),
(iv) Lactobionic acid (4β-galactosido-gluconic acid),
(v) Tetragalacturonic acid[1] (+$CaCO_3$ when sterile),

and the following other substances; gum arabic, mesquite gum, glycerol, and starch. The medium in all cases but the last was a broth containing 1 per cent. carbohydrate, 0·1 per cent. peptone, 0·05 per cent. ammonium phosphate. The starch was used in an agar medium of similar composition on plates, the better to observe any zoning round the colonies.

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[1] I am indebted to Prof. K. P. Link for this acid.

The growth on these carbohydrates is given in Table II. It will be seen that glycerol, starch, and the two gums, were fermented by all the organisms. Similarly tetragalaturonic acid was utilised by all, though not to any great extent. This was not unexpected, inasmuch as pectin, of which molecule tetragalaturonic acid forms about 80 per cent., was previously shown to be available.

Gluconic acid, the normal monocarboxylic acid from glucose, was fermented by all organisms, but saccharic acid which has a carboxyl group at each end of the hexose unit was unavailable to all but organisms 8 and 17. These two organisms, however, along with some others, could not utilise maltobionic acid. Lactobionic acid, for no obvious reason, appeared to be more available than maltobionic acid.

Table II.

Utilisation of carbohydrates by mesophilic organisms.

	Gluconic acid		Saccharic acid		Maltobionic acid		Lactobionic acid		Tetra-galaturonic acid		Gum arabic	Mesquite gum	Glycerol		Starch agar
Organism	1 day	7 days	2 days	11 days	2 days	11 days	1 day	5 days	4 days	10 days	3 days	2 days	1 day	11 days	1 day
1	–	+s	–	–	–	–	+	+p	?	+	+	+	+	+	++*
2	–	+ps	–	–	–	–	–	+p	+	+	+	+	+	+	++†
3	–	+ps	–	–	–	+	–	+p	+	+	+	+	+	+	++†
4, 6, 12, 14, 15	+	+ps	–	–	–	+	+	+	+	+	+	+	+	+s	‡
5	–	+ps	–	–	+	+	+	+	+	+	+	+	+	+ps	++†
7	–	+ps	–	–	–	+	+	+	+	+	+	+	+	+	++*
8	–	+s	+	+	–	–	–	+	+	+	+	+	+	+	+*
9	–	+p	–	–	–	–	–	–	+	+	+	+	+	+	+†
10	–	+ps	–	–	–	–	–	+	+	+	+	+	+	+	++†
11	–	+p	–	–	–	–	+	+p	+	+	+	+	+	+	+*
13	–	+p	–	–	–	+	–	–	+	+	+	+	+	+	+*
16	–	+p	–	–	–	–	+	+p	+	+	+	+	+	+	++†
17	–	+p	+	+	–	–	+	+	+	+	+	+	+	+s	+†

+ =growth; ++ =heavy growth; *p* =pellicle; *s* =sediment; * =clear zone round colony.

‡ 4 and 6 fair growth, no adjacent clearing; 14 and 15 fair growth, some clearing; 12 fair growth, good clearing.

Identification of organisms.

The main purpose of this work was to isolate certain organisms capable of utilising hemicelluloses, to determine their activity and general fermentation characteristics, but not necessarily to assign to them a permanent classification. In any case, from the evidence obtained, this would be no easy matter, inasmuch as certain of those isolated do not appear to have been previously described, or else fall in a class in which differentiation is difficult. One group, composed of organisms 4, 6, 12, 14 and 15, stands out from the remainder. These were small rods, Gram-negative, giving no pigment, and forming no spores. They agree closely with the published description of *Achromobacter ubiquitum* (Jordan) originally described as *Bacillus ubiquitus* (2). Several organisms with very similar characteristics have been named separately in this group. The five strains isolated were not identical but showed small morphological and cultural differences notably in growth on potato, and on starch agar plates, and in the strength of the catalase test. Organism 17 possibly falls in the *Achromobacter* group also, though rather above the average size. It does not, however, produce gas from carbohydrates as do those five strains mentioned above.

The remaining organisms were aerobic spore-formers, so common in soil. Not all were Gram-positive, however, and it is possible that the Gram-negative forms represent new species. The cellulose-decomposing organisms of this group, such as *B. amylolyticus* and *B. albus* are Gram-negative and both ferment a rather wide range of carbohydrates producing acid but no gas, as do those isolated in this work. This class of organism deserves further attention.

The thermophilic organisms were not so completely characterised, but in any case identification of members of this group is a difficult and unsatisfactory matter. They exhibited marked pleomorphic characters, so that their purity was not a matter of absolute certainty. Plectridial forms were sometimes found in culture X, while Z had some of the characters of an actinomycete.

Fermentation experiments.

The organisms described were frequently transferred on to media containing hemicellulose lest there should be any loss of activity. While the mixed enrichment cultures from which these were isolated produced a rapid loss of hemicellulose from liquid media, the same could not be said of any of the organisms in pure culture. On media such as HAL or HBL, the faster organisms showed some signs of clearing of the medium at the end of two days. The complete clearing of the opalescent solution was frequently not accomplished in a month, if at all. Various fermentations were carried out with the organisms which seemed to be the more active and rapid. These were notably 3, 7, 8, 13, 16 and 17. The rate of fermentation of a purified sample of hemicellulose A from oat straw by these organisms was determined. To a suspension in water was added per litre: peptone 1 gm., ammonium phosphate 0·5 gm., potassium phosphate 0·5 gm. and a trace of iron. On autoclaving, an opalescent solution was obtained. About 400 c.c. were fermented at 30° C. in flasks with a side tube which permitted sampling from time to time. At the end of 8, 24 and 40 days about 80 c.c. were withdrawn. Two samples of 10 c.c. were centrifuged to remove sediment, and the residual hemicellulose in each precipitated by the addition of three volumes of alcohol to which a little acetic acid had been added. The precipitate was filtered off, thoroughly washed, and weighed after drying in a vacuum oven at 60° C. and then briefly at 100° C. The assumption that the alcohol precipitate consisted of undecomposed or only slightly degraded hemicellulose is justifiable since the furfural yield of the fermenting solution (which was determined on two 20 c.c. portions) and of the precipitate fell approximately proportionately. In Table III is given the decomposition effected by the organisms selected.

Table III.

Mg. alcohol precipitable hemicellulose in 10 *c.c. fermentation liquid. Initial concentration* = 81 *mg.*

Days	No. 3	No. 7	No. 8	No. 11	No. 16	No. 17
8	72	71	80	74	76	77
24	70	68	76	74	75	74
40	64	66	72	73	73	73

In no case was more than 20 per cent. of the hemicellulose fermented. A mixed culture from soil, on the other hand, in 40 days, left only 8 mg. in a solution initially of the same concentration, a removal of about 90 per cent.

It was mentioned previously that although a wide range of carbohydrates was utilised by these organisms very little acid was produced, and only small quantities of gas, save in the case of those of the *Achromobacter* group. Inasmuch as arabinose and galactose are frequently found in plant hemicellulose, the fermentation of both these sugars by the organisms given above was tested. The residual sugar was determined by the Shaffer-Hartman method, the initial concentration in each case being 1 per cent.

Table IV.

Percentage residual sugar after 2 *weeks' incubation at* 30° *C.*

	No. 3	No. 7	No. 8	No. 11	No. 16	No. 17
Arabinose	0·91	0·97	0·96	0·97	1·0	1·0
Galactose	0·91	1·0	0·97	0·96	0·94	0·97

This experiment demonstrated again the apparently feeble nature of these organisms in pure culture. On pectin, however, a rather greater activity was observed. This was somewhat unexpected, since the units commonly occurring in the hemicelluloses are similar to those in pectin. The linkages of the latter, however, must be of an entirely different nature, inasmuch as they are extremely sensitive to alkaline degradation, while the hemicelluloses are comparatively resistant to alkali. Hydrolysis studies have shown that a solution of pectin, degraded so that no calcium pectate is obtained by the usual method of estimation, may even yet contain polyuronide molecules of considerable size and complexity. Therefore, if in a fermentation trial all pectin is removed, it must not be assumed that all carbohydrate material has been fermented away. In these experiments a solution of pectin to which the usual inorganic salts had been added was sterilised before adjustment of the *p*H[1]. Calcium carbonate was added subsequently and inoculations made from broth. At the end of 14 days the cultures were acidified with acetic and hydrochloric acid to remove calcium carbonate. After neutralisation with NaOH and filtration, the liquid was acidified with acetic acid and heated with an excess of calcium chloride. The calcium pectate was filtered off, washed and weighed. In Table IV is given the pectin remaining in a series of tubes, the pectin being expressed as calcium pectate.

Table V.

Mg. of pectin remaining after fermentation at 30° *C., for* 14 *days, solution initially contained* 327 *mg.*

Organism		Organism		Organism	
1	0	8	92	12	242
2	0	9	20	13	85
3	0	10	88	16	6
5	124	11	100	17	82

It will be seen that there is considerable variation in pectin-decomposing ability, but this does not necessarily imply that there is great variation in the actual quantity of carbohydrate fermented, as it is only the intact pectin ring which is being estimated.

These fermentation tests having shown that the activity of the organisms isolated is not very great in pure culture and on purified substrates, it was thought worth while to test their action on straw. 15 gm. portions were autoclaved in small flasks, and 50 mg. nitrogen added to each in the form of a sterile solution of peptone or ammonium phosphate.

[1] The natural *p*H of pectin solution is about 4·0. If this be adjusted to 7·0 or thereabouts before sterilisation, a very considerable loss will occur. A pectin solution brought to *p*H = 7·2 and autoclaved for 15 minutes at 121° C. yielded only 59 per cent. calcium pectate.

The inoculations were made by adding 10 c.c. from a glucose-soil extract broth in which the organisms were actively growing, and in addition 85 c.c. water was added. After incubation at 30° C. for 1 month the total loss was determined. Inasmuch as this was in all cases small, no analyses for constituents were made.

Table VI.

Fermentation of oat straw with various organisms at 30° C. Loss per cent. of straw in one month.

	Organism					
N source	1	7	8	12	16	17
Peptone	5·0	—	4·1	3·0	5·2	5·7
Ammonium phosphate	—	0·5	5·3	2·5	4·9	0·9

In the same period a mixed inoculum from an enrichment culture in hemicellulose medium caused a loss of 14·5 per cent. of the straw when supplied with organic nitrogen. It is clear that the type of organism isolated is not capable alone of bringing about any general decomposition of a plant material such as straw, or even of fermenting to any considerable extent the 20–30 per cent. of hemicellulose contained in straw.

III. DISCUSSION.

The purpose of the work described in the preceding pages was, by isolating some of the responsible organisms, and testing others, to account for the observed phenomenon that hemicelluloses are rapidly and often almost completely removed in the natural decomposition of plant materials under aerobic conditions. That some fungi in pure culture can achieve such a removal has been demonstrated unquestionably by Waksman (6) and Norman (3). The latter showed that some common Aspergilli would remove in pure culture practically as much hemicellulose as a mixed natural flora in the same time. Many of those fungi that have been isolated from such decompositions attack cellulose also, and do not seem, therefore, to be particularly specific to any one plant constituent.

Nevertheless it was not anticipated that all of the fungi tested would be able to utilise a purified hemicellulose as the sole carbohydrate source. Certain of the organisms chosen are not normally found in the soil. It would seem that the ability to utilise hemicellulose is widely distributed and common amongst fungi. The test, as made, was purely qualitative and gave no indication as to the extent or rate of utilisation. Nevertheless, in many cases there was a very obvious production of an exo-enzyme hydrolysing hemicellulose, and therefore a strong probability that fermentation would be rapid.

In the case of bacteria the situation is rather different, for no organisms have been described which in pure culture can validly be described as very active in decomposing this group of polysaccharides. Of the large number of aerobic organisms isolated by Waksman and Diehm (9) none completely fermented the polysaccharide preparations upon which they were tested, and in the case of xylan, which corresponds most closely to the plant polyuronides here considered, only three organisms produced as much as 50 per cent. fermentation of a dilute solution (0·15 per cent.) in 6 weeks. This cannot be regarded as a rapid utilisation, nor, on this evidence, is it likely that such organisms play any important part in a natural decomposition in which the hemicelluloses may be more or less completely removed in a few days.

In all, it appears that while in soil and in manure there are bacteria of various types which are capable of attacking the hemicelluloses, they are relatively feeble in fermentative power, at least when tested on the purified polysaccharides. Furthermore, some of the organisms are not really active in utilising common sugars, producing little of either acid or gas. There is the possibility that the purified polysaccharide may be less available than the natural material, as has been suggested by Waksman and Diehm (9). This raises again the question as to whether or not availability tests are not too drastic. Cellulose or starch decomposition is determined by giving the organism cellulose or starch as the sole source of energy. It has been shown that this is sometimes too rigid a condition, and that certain organisms when actively developing on a particular energy source, can, at the same time, cause decomposition of another substance upon which they are unable to develop alone. This is particularly true of certain fungi which will not grow upon cellulose agar plates, but which can be shown by analysis to be excellent decomposers of cellulose when growing upon a material such as straw. Similarly Shrikhande (5) recently demonstrated that *Mycobacterium agreste* which had not been known to attack cellulose, or grow on cellulose plates, does in fact produce a very considerable decomposition of the cellulose of straw, and is a more active cellulose decomposer under these conditions than the well known organism *Spirochaeta cytophaga* (*Cytophaga Hutchinsoni*). Nevertheless there is evidence in the case of some of the organisms isolated in this work, and some of those obtained by Waksman and Diehm (9) that the test of availability as applied on the purified hemicellulose is not too rigid, since when inoculated on to natural materials, such as corn cobs or straw, they were not appreciably or considerably more active.

There is the further possibility that these organisms may be more effective when working in association, and it is unquestionably time that crude enrichment cultures produce a more rapid decomposition than do any of the organisms alone. This may be due to the presence of secondary organisms utilising sugars or other degradation products produced by the organism actually attacking the hemicellulose. Whether that is so must be decided by further investigation, but the fact remains that a search for bacteria in soil and manure which can actively attack and decompose the polyuronide type of hemicellulose has not been successful, though a number of organisms have been obtained which are rather feeble in their action in pure culture. These organisms are of common soil types, but can utilise an unusually wide assortment of sugars and polysaccharides. Indeed no sugar was found to be unavailable, and differences were only observed by supplying to them sugar acids not likely to occur naturally.

Considering therefore this problem in its wider aspects, it seems probable that the ability to ferment hemicelluloses will be found to be a general property of common soil fungi, in contrast to the rather restricted fermentation by bacteria, and that to the fungi must be ascribed a prominent rôle in the earlier stages of the decomposition of plant materials containing hemicelluloses.

IV. SUMMARY.

1. The decomposition of the hemicelluloses of plant materials occurs rapidly under normal aerobic conditions. Since a number of fungi have been shown by analyses of pure culture rots to be active in fermenting this group, as well as cellulose, some seventy common fungi, mostly Aspergilli and Penicillia, were tested as to their ability to utilise on agar plates the crude hemicellulose from oat straw.

2. All of the fungi tested grew reasonably well on the hemicellulose medium, the majority at least as well as on glucose, and a number better than on that sugar. In some cases there was production of a cleared zone round the colony due to an exo-enzyme. It is likely that ability to ferment hemicelluloses will be found to be a general property of common fungi.

3. From soil, manure, and straw, twenty aerobic bacteria were isolated capable of utilising hemicelluloses. Three of these were thermophilic

forms. Their morphological characters and biochemical reactions were determined.

4. All the organisms utilised a very wide range of sugars and polysaccharides; one group produced some acid and gas, the remainder little. Differentiation was obtained on saccharic acid and certain disaccharide acids.

5. Five of the mesophilic organisms agreed closely with the description of *Achromobacter ubiquitum* (Jordan). The remainder were aerobic spore-formers, though not all Gram-positive. The negative forms may represent new species.

6. None of the organisms in pure culture could validly be described as active in decomposing the isolated hemicellulose, or that *in situ* in straw.

7. Fungi seem to be more active and important in the natural decomposition of hemicelluloses than bacteria, unless when working in association the rather restricted individual fermentative powers of the latter are considerably enhanced.

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EFFECTS OF TEMPERATURE AND HUMIDITY ON THE CLOTHES MOTH LARVA, *TINEOLA BISELLIELLA* HUM. (LEPIDOPTERA)

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INTRODUCTION.

THE larva of the clothes moth is known to be able to survive starvation for considerable periods (7), and its metabolism has been shown to be influenced by the climatic conditions of the environment (8, 9). This paper describes work in which larvae were subjected to a number of different conditions of temperature and humidity, and the effects on their metabolism studied. These experiments continue work which has already been done on other insects (1, 3, 4, 5), and the main interest of the results obtained from the clothes moth larva is in comparing them with those obtained from different species. When more insects have been studied in a similar manner, it should be possible to produce a clear picture of the way in which the group as a whole responds to the climatic factors in its environment.

(*a*) *Breeding.*

The clothes moths were bred in glass jars of about 2½ litres capacity, covered with phosphor-bronze gauze. The jar was half-filled with broken cattle cake on which the larvae fed, and among which pupae were formed. When the adults emerged they lived in the space above the food. The confinement did not appear to affect their vitality, as they readily paired and laid many fertile eggs. The culture was kept at a temperature of 22° C., where the insects bred continuously without requiring any attention.

(*b*) *Experimental technique.*

The larvae for use in the experiments were picked out by hand from the food, and put separately into 3 × 1 in. glass tubes. The tubes were covered with cotton gauze. The larvae did not feed on the cotton, but a few bit through the gauze and escaped. These were probably larvae which were ready to pupate (8). Larvae were not selected at any particular stage in their development, as the length of larval life and the size at which pupation takes place is so variable. The clothes moth has not a definite number of larval instars (10), and apparently normal pupae vary in weight between 3·5 and 9·5 mg. (8). The larvae used in the experiments varied between 3 and 12 mg., and their size did not appear to affect the results in any way.

Before starting an experiment, the larvae were kept isolated for two days. This was done because previous work showed that when mealworms (*Tenebrio molitor*) were removed from their food, they passed considerable amounts of excreta during the first two days and practically none later on. This was not the case with the clothes moth larvae; although they passed a considerable quantity of excretion during the first two days, they excreted at intervals during the whole experimental period. For the experiments, the larvae (in the tubes) were kept in desiccators containing different mixtures of sulphuric acid and water to control the humidity (11). The desiccators were kept in incubators at several temperatures. The larvae were weighed on a torsion balance, which was accurate to 1/40 mg.

(*c*) *Analysis of unstarved larvae.*

Individual larvae were dried at 105° C. and the proportion of dry matter found. Experiments were made in February 1932 and 1933, and in both years the proportion of dry matter was found to be 40·7 per cent. of the live weight (standard error 0·37); forty-eight larvae were used. The proportion of dry matter of thirty-five larvae from the same culture in September 1932 was 43·3 per cent. (standard error 0·34), which is significantly higher than the result given above. The reason for this is not known. The larvae used in September were not larger than those used in February, and there was no significant correlation between larval size and proportion of dry matter. The results of each experiment are compared with controls taken from the culture at the same time.

(*d*) *Loss in weight.*

The clothes moth larvae were exposed to four different temperatures: 35, 30, 22 and 10° C. and at each temperature to air with a relative humidity of 0, 30, 60 or 90 per cent. As it was desired to study larvae in a healthy condition, they were subjected for periods short enough to ensure that very few died. This meant that at the higher temperatures the experiments lasted over shorter periods than at the lower. At 35° C. the larvae were exposed for 7 days, and at 10° C. for 28 days.

The results obtained are summarised in Table I. The insects lost weight more rapidly in dry air than in moist, and at high temperatures than at low. At each temperature, the amount of dry matter present in the insects at the end of the experiment, expressed as a percentage of the original weight, was the same no matter to what humidity they had been exposed. This means that the same proportion of dry matter was lost at each humidity. Dry matter was lost due to oxidation of reserves, to excretion and to silk production. The weight of silk formed was very small indeed, and can be neglected. As the excretory products were produced in minute amounts over the whole experimental period, it was not found possible to determine exactly the proportion of dry matter lost due to this cause. Rough analysis indicated that rather more than half the dry matter which disappeared was excreted, except at 10° C. where the proportion was lower. At each temperature, the rate at which reserves were metabolised was probably not affected by the humidity. If it had been, it would mean that at those humidities where less food reserves were used, excretion was increased, with the result that the same gross weight of dry matter was lost as at other humidities where the rate of metabolism of reserves was higher (and the rate of excretion proportionately lower). It seems much more likely that at each temperature both the rate of metabolism and the rate of excretion were not affected by humidity.

(*e*) *Water balance.*

Reference to Table I shows that the proportion of water in the bodies of the larvae after starvation was different from that at the start of the experiments. In all cases, subjection to a humidity of 0 per cent. caused an increase in the proportion of dry matter, while a humidity of 90 per cent. caused an increase in the proportion of water. Now it has been indicated that the same amount of excretion and metabolism occurred in dry or moist air (at one temperature), so the upset in the water balance must have been caused by a difference in the amount of water evaporated.

When working with the mealworm and the bed-bug (4, 5), it was shown that any loss in weight in the insects during starvation was due to water evaporated. That is not the case with the clothes moth. In the mealworm and the bed-bug, evaporation and metabolism are the only factors changing their mass and composition. Metabolism does not alter the gross weight of either of these species. I have not made any extensive analyses of starved and unstarved clothes moth larvae, but it is probably safe to say that as they are using mainly fat reserves, if there is any change in gross weight due to metabolism it will be negligible in comparison with the change caused by excretion or evaporation. Therefore it may be taken that any loss in weight other than that caused by excretion is due to evaporation.

Table I.

No. of larvae								
End	Start	Days starved	Temp. ° C.	R.H. %	Weight at end as % original weight	Dry matter as % end weight	Dry matter as % original weight	% dry matter in control
26	34	7	35	0	*75·7* ±1·41	*42·8* ±0·86	*32·2* ±0·61	40·2 ± 0·37
13	13	—	—	30	*81·6* ±2·02	*39·6* ±0·69	*32·2* ±0·90	
11	14	—	—	60	*83·4* ±1·64	*37·76* ±0·60	*31·83* ±0·58	
26	28	—	—	90	*85·8* ±1·26	*38·2* ±0·77	*32·7* ±0·64	
10	15	7	30	0	*75·3* ±2·49	*46·5* ±1·91	*34·3* ±0·60	43·3 ± 0·34
13	15	—	—	30	*77·0* ±2·26	*45·7* ±2·18	*34·7* ±1·06	
13	16	—	—	60	*81·3* ±1·70	*43·3* ±0·94	*35·0* ±0·49	
10	12	—	—	90	*85·3* ±2·10	*40·4* ±0·57	*34·3* ±0·54	
6	12	14	22	0	*73·1* ±2·37	*42·4* ±2·37	*30·8* ±0·80	40·7 ± 0·37
4	8	—	—	30	*78·4* ±3·47	*39·5* ±1·26	*31·0* ±1·33	
5	7	—	—	60	*75·0* ±4·30	*42·0* ±4·5	*31·6* ±2·1	
13	18	—	—	90	*80·6* ±1·38	*37·0* ±0·41	*30·0* ±0·69	
(i)								
18	18	28	10	0	*82·3* ±0·95	*43·3* ±0·57	*35·3* ±0·58	43·3 ± 0·34
17	19	—	—	30	*84·8* ±0·74	*41·5* ±0·66	*35·4* ±0·62	
18	20	—	—	60	*86·2* ±0·72	*42·8* ±0·53	*36·8* ±0·47	
18	20	—	—	90	*91·0* ±0·82	*41·3* ±0·38	*37·6* ±0·45	
(ii)								
15	20	—	—	0	*87·0* ±1·26	*42·2* ±0·75	*37·0* ±0·60	40·7 ± 0·37
19	20	—	—	90	*94·3* ±0·83	*38·8* ±0·52	*36·3* ±0·48	

(i) and (ii) done in September and February respectively. The standard error is given following each number.

It appears that the rate of evaporation of water from many insects is directly proportional to the saturation deficiency of the air (2, 3, 4, 5, 6). Is this also the case with clothes moth larvae? It has been shown that the loss in weight due to excretion, etc., at any temperature is not affected by the humidity of the air. The amount of water evaporated is equal to the total decrease in weight during the experiments minus the weight of excretion and silk produced. As the exact weight of the silk and of the excretory substances is unknown, the amount of evaporation must be estimated indirectly.

Consider the results in Table I obtained at 35° C. In air with a relative humidity of 90 per cent., the final weight was 85·8 per cent. of the original. With 0 per cent. humidity, the final weight was 75·7 per cent. Now the same amount of excretion took place in each case. Therefore the difference between 85·8 and 75·7 represents the excess of evaporation (from 100 unit weight of larva) when the air has a relative humidity of 0 per cent., over that which takes place in air with a humidity of 90 per cent.

Now if the rate of evaporation of water from the clothes moth larva is proportional to the saturation deficiency of the air, then the expression

$$\frac{\text{Weight of water evaporated}}{\text{Saturation deficiency}} \times \frac{1}{\text{Time}}$$

should be constant for all temperatures. We do not know the total evaporation under any conditions, but we know the excess evaporation in air with a humidity of 0 per cent. over air with a humidity of 90 per cent. If the rate of evaporation is proportional to the saturation deficiency, then the expression

$$\frac{\text{Excess of evaporation at 90 per cent. humidity over that at 0 per cent.}}{\text{Difference between saturation deficiency and 0 and 90 per cent. humidity}} \times \frac{1}{\text{Time}}$$

should be constant. This factor has been worked out for the figures in Table I, and the results are given in Table II. The numbers obtained vary only between 0·301 and 0·384, with the exception of the result obtained at 30° C., which gives a figure of 0·501. It is interesting to note that the factors calculated from the results of the two experiments made at 10° C. agree fairly closely with one another—the differences in Table I are due to differences in excretion.

Table II.

Temp. ° C.	Weight at end as % original weight 90 % R.H.	Weight at end as % original weight 0 % R.H.	Difference	Difference between saturation deficiency at 90 and 0 % R.H.	Time in days	$\frac{F}{S} \times \frac{1}{\text{Time}}$
35	85·8	75·7	10·1	37·6	7	0·384
30	85·3	75·3	10·0	28·5	7	0·501
22	80·6	73·1	7·5	17·8	14	0·301
10	91·0	82·3	8·7	8·3	28	0·375
10	94·3	87·0	7·3	8·3	28	0·314

$$\frac{F}{S} = \frac{\text{Final weight at 90 \%—Final weight at 0 \%}}{\text{Saturation deficiency at 90 \%—Saturation deficiency at 0 \%}}.$$

Taken by themselves, the figures in Table II are sufficiently close to one another to *suggest* that the rate of evaporation of water from the clothes moth larva is proportional to the saturation deficiency of the air, but they cannot be said to prove it. The original data are very variable, so it is possible that the agreement between four out of five of the numbers may only be a coincidence. But when the results obtained from other insects are considered, it appears likely that the clothes moth larva is behaving in the same manner as they are, and more significance can be attached to the results. The mealworm is able to keep the proportions of dry matter and water constant in its body in dry air, but in moist air its water balance is upset. The opposite occurs with the bed-bug, which maintains its water balance in moist but not in dry air. The clothes moth appears in some ways to be intermediate, for while its water : solid ratio remains constant during experiments in moderately dry or moderately moist air, the ratio does not remain constant in very dry or very moist air. In dry air, it loses water more rapidly than it produces it by metabolising reserves. In moist air, metabolic water is produced more rapidly than it can be evaporated. The insect has no control over the rate of metabolism, and therefore over the rate at which water is produced in its body, nor has it any control over the rate at which the water formed is lost by evaporation. Therefore its water balance is upset in either very dry or very moist air.

SUMMARY.

Methods for breeding clothes moths with very little attention are described.

Experiments were made in which fasting clothes moth larvae were exposed to a number of temperatures (35, 30, 22 and 10° C.), and four humidities at each temperature (0, 30, 60 and 90 per cent.). The rate of loss of weight of the larvae was determined, and also the amount of solid matter lost.

In moderately dry or moderately moist air, the larvae maintained their water balance, but in very dry air the proportion of solid matter in their bodies rose, and in moist air it fell. A considerable amount of dry matter was lost by excretion and the production of silk, in addition to metabolism. These processes were not affected by humidity.

The results suggest that the rate at which water is evaporated from the larvae is proportional to the saturation deficiency of the air.

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ON THE BIOLOGY OF THE MITE, *GLYCYPHAGUS DOMESTICUS* DE GEER. (TYROGLYPHIDAE, ACARINA)

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The importance of the occurrence of *Glycyphagus* in houses has long been recognised, and many records exist of the appearance of the mite in large numbers on furniture and in stored foods of many kinds. No systematic attempt has been made, however, to study the habits and life history of this mite in detail and to publish the results in a form which would be generally accessible to the general public and to the furniture industry. The most illogical views are held by many people about mites in general and *Glycyphagus domesticus* De G. in particular, and this has sometimes led to unjust claims against the furniture trade and to legal cases which could easily have been avoided.

Some years ago a pamphlet (1) which is still available was issued by the Natural History Museum, and although this did a great deal towards clearing up the mystery surrounding mites, it was obvious that there were still considerable gaps in our knowledge of the biology and ecology of *G. domesticus*.

LIST OF MITES FOUND IN HOUSES.

The following mites frequently occur in houses:

A. *Family Tyroglyphidae.* Mites of this family are commonly found in all kinds of stored food products where they often occur in large colonies and do a considerable amount of damage. They are relatively simple in structure and are characterised by: (1) the soft, pale-coloured body with a strut-like supporting skeleton; (2) the absence of specialised sense organs and respiratory system; (3) the possession of simple chelate chelicerae which work perpendicularly and are almost non-retractile; and (4) the occurrence of a resting or hypopial stage in the life-history.

In this family several species commonly occur in houses:

(1) *Glycyphagus domesticus* De Geer—the furniture mite—on green Algerian fibre, yeast, dry fodder, dried fruits, cheese and any kind of mouldy substance.

(2) *Glycyphagus cadaverum* Schrank—closely allied to *G. domesticus* and frequently found on the same substances.

(3) *Carpoglyphus anonymus* Haller—on dried fruits, decaying nuts, cheese, jam and wine dregs.

(4) *Aleurobius farinae* De Geer—on straw packing, flour and any dried cereals.

(5) *Glycyphagus plumiger* Koch—on green Algerian fibre, hay and dust in warehouses.

(6) *Tyroglyphus longior* Gervais—the cheese mite—on decaying Algerian fibre, cheese and any mouldy cereals.

(7) *Tyroglyphus heterocomus* Michael—on decaying Algerian fibre.

(8) *Histiogaster entomophagus* Laboulb.—on vinegar dregs and flour.

The mass increase of Tyroglyphid mites is kept in check to some extent by larger predaceous mites. The majority of these belong to the family Cheyletidae which may be distinguished from the Tyroglyphidae by: (1) the possession of a well-developed tracheal respiratory system opening by definite stigmata; (2) the modification of the chelicerae to form stylets used for piercing and sucking; (3) the enlargement of the pedipalps to form a clasping organ; (4) the parthenogenetic form of reproduction.

The two commonest species are:

(1) *Cheyletus eruditus* Schrank.

(2) *Cheyleteomorpha venustissima* Koch.

Both of these species are larger than the Tyroglyphidae upon which they feed; they do not provide any adequate check on them, however, as the Cheyletids tend to eat each other when the numbers of Tyroglyphidae become reduced.

Glycyphagus domesticus is the most common species found infesting furniture and has been chiefly studied in this investigation.

OCCURRENCE OF *GLYCYPHAGUS DOMESTICUS* IN HOUSES.

It is very probable that small numbers of this mite are present in all houses feeding on minute particles of organic debris and kept from excessive reproduction by adverse hygrometric (dry) conditions. If left undisturbed, however, in favourable conditions and on suitable food, they increase to very large numbers in a comparatively short space of time. In dwelling houses they frequently occur in masses on preserved fruits, yeast or stored puddings, etc., but they are a greater source of trouble when they start breeding inside upholstered furniture. At first they remain unnoticed, but if a spell of dry weather sets in, the mites leave the interior of the furniture and swarm over the whole room. In bad infestations, the mites congregate to form a powder-like mass which covers all available surfaces and ultimately spreads over the whole house. These moving masses although harmless are objectionable and particularly repulsive to some people; their presence is known to have caused nervous breakdowns in sensitive individuals.

Bad cases of mite infestation are liable to occur in dark, damp and rarely used rooms, particularly in newly built houses. The mites breed specially readily in heavily upholstered furniture, covered with hide and stuffed with untreated green Algerian fibre. Such a suite of furniture if kept in an unused damp room is little affected by slight fluctuations in atmospheric conditions.

A period of wet, warm weather is favourable to rapid multiplication.

Glycyphagus domesticus has been recorded by Michael (2) as infesting rush furniture in large numbers, and Oudemans found the same thing at the Hague where, he says, the Acari literally covered the furniture of the whole house and were feeding on the animal fast adhering to the incompletely cleaned horsehair with which the furniture was stuffed.

SOURCE OF INFECTION.

It is often impossible to determine the source of infection of an outbreak of furniture mite. The mites are so small that they may be present in houses for many years without being noticed: in fact, it is extremely likely that a few are present in every house at all times. In many instances, it is certain that the fibre is infected before being used for stuffing, as *G. domesticus* is present in warehouses at the docks where the fibre is temporarily stored. Infection also probably occurs in the holds of vessels in which the fibre is transported from Algeria and Morocco. In very damp houses, even though they contain no furniture stuffed with Algerian fibre, the mites may increase to noticeable numbers as they are able to feed on mould fungi growing on wallpaper, etc.

LIFE HISTORY OF *GLYCYPHAGUS DOMESTICUS*.

The adults pair after emergence from the deutonymphal skin, and clusters of eggs are laid about a week later. The eggs are at first opaque but become transparent as the yolk is absorbed. The six-legged larvae emerge after a five-day incubation period; they are similar to the adults in general appearance but have fewer hair-like appendages and possess a pair of suckers on the ventral surface at the level of the second pair of legs. They are at first wrinkled in appearance but ultimately become swollen and glassy. The larvae, at this stage, cease feeding, wedge themselves into crevices and pass into a resting condition. After resting, the skin is cast and the protonymph emerges; this is very like the larva but possesses an extra pair of legs. Ultimately this first nymph also passes into an inert condition and gives rise to the deutonymph. This is much larger than the two preceding stages and possesses rudiments of external genitalia, although it is impossible to distinguish between the two sexes. Males and females emerge from the resting deutonymphal stages and pair almost immediately. The following is a summary of the life cycle under almost optimum conditions, *i.e.* at 25° C. and a relative humidity of 90 per cent.:

Period of incubation of egg	5 days
Active larva	2 ,,
Resting larva	2 ,,
Active protonymph	4 ,,
Resting protonymph	2 ,,
Active deutonymph	5 ,,
Resting deutonymph	2 ,,
Total length of life cycle	22 ,,

The hypopial stage.

In a certain percentage of individuals the life history is complicated by the intercalation of an extra stage—the hypopus—which replaces the resting protonymph. This hypopial stage occurs in many species of Tyroglyphidae and usually takes the form of a flattened, well-chitinised animal, which by means of a number of suckers projecting through a plate in the anal region is enabled to cling to passing animals and thus may be transported from one locality to another. The hypopial form of *G. domesticus* was first described by Michael; it is a small cyst-like body devoid of appendages which remains enclosed within the shrivelled protonymphal skin. In the course of its formation a considerable amount of reorganisation occurs within the body of the mite which is closely correlated with the loss of movement and power of feeding. The internal organs become concentrated at the end of the body and a constriction appears posterior to the fourth pair of legs. The mite finally ceases to move and becomes wedged with its head fixed into a crack, only the posterior end projecting. This end becomes flattened, the flat surface splitting along the ventral and lateral sides so as to form a trap door leading to the enclosed hypopus. The internal changes that occur within the hypopial skin are the complete degeneration of the muscular system into blocks of cells, its place being taken by parenchymatous tissue, the closing of the mouth and anus, the rest of the gut becoming a solid block of cells, and the checking of the development of the reproductive system. The nervous system remains unchanged.

The hypopial stage remains encysted for various periods of time ranging from five days to six months, and the deutonymph will only start to develop when a suitable temperature and humidity have been attained. In most species of Tyroglyphidae the hypopus occurs sporadically, but in *G. domesticus* as many as 50 per cent. of the mites may pass through this stage. Table I shows the number of offspring from one pair of mites that pass through the hypopial stage, the number of eggs laid by one female, and the rate of egg-laying.

A considerable number of opinions have been published as to the reason for the occurrence of the hypopial form in the life history of the Tyroglyphidae, and the subject is discussed at some length in Michael's Monograph on the *British Tyroglyphidae*, pp. 143–73.

In the course of this investigation it has become clear that:

(1) The hypopus is a resistant form which will enable the species to survive during adverse conditions.

(2) The cause of its formation is unknown.

(3) In *G. domesticus* it is not formed as the result of adverse conditions.

(4) Nor as the result of overcrowding.

(5) It is only formed at one phase in the life-cycle, *i.e.* between the proto- and deuto-nymphal forms.

(6) It gives rise to both males and females which are exactly similar in appearance to the adults produced after a direct development.

(7) The occurrence of the hypopial form does not affect the fertility of the adult.

THE FOOD OF *GLYCYPHAGUS DOMESTICUS*.

G. domesticus can be kept alive and reared successfully on many substances, particularly those that readily absorb and retain water when placed in a moist atmosphere. Plant and animal tissue thus become suitable media for the growth of the mould fungi which form the staple food of the Tyroglyphidae. The mites crop the mycelium which they are capable of digesting and also eat the fungal spores which pass unharmed through the body and are discharged in the faeces.

Table I.

Life history of Glycyphagus domesticus.

Date ...	Feb. 7	Feb. 8	Feb. 9	Feb. 10	Feb. 13	Feb. 14	Feb. 15	Feb. 17	Feb. 20	Feb. 21	Feb. 22	Feb. 27
No. of eggs laid ...	4	1	6	6	25	9	8	7	10	5	2	3
Feb. 13	2 RL, 1 L	1 RL	—	—	—	—	—	—	—	—	—	—
,, 14	1 N1, 2 RL	1 RL	—	—	—	—	—	—	—	—	—	—
,, 15	3 N1	1 N1	6 L	—	—	—	—	—	—	—	—	—
,, 17	3 N1	1 N1	2 RL, 4 L	6 L	15 L	—	—	—	—	—	—	—
,, 20	2 H, 1 N1	1 H	1 RL, 5 N1	6 N1	11L, 4 N1, 10 RL	9 L	5 L	—	—	—	—	—
,, 21	1 RN1	—	6 N1	5 N1, 1 RN	L, RL, N1	9 L	8 L	2 L	—	—	—	—
,, 23	1 N2	—	5 N1, 1 Rn	1 N2, 5 N1	4 N1 etc.	6 RL, 1 N1	4 RL, 3 L	3 RL, 3 L	—	—	—	—
,, 27	1	—	4 N1, 2 N2	2 H, 3 N1, 1 N2	—	7 N	6 N1, 2 RN1	1 RN1, 5 N1	4 L, 1 N1 2 RL	4 L	—	—
Mar. 1	—	—	—	—	16 N 1 Rn	7 N	5 N, 1 RN	1 RN1, 4 N	2 RL, N	3 L, 2 N	—	—
,, 2	—	—	1 , 1 RN2 4 H	4 N2, 2 H	7 N, 10 N1, 3 H	4 N, 3 H	4 N, 3 H, 1 RN1	4 N, 1 RN2	—	—	1 L, 1 R1	—
,, 3	—	—	1 , 1 ,	1 Rn2, 3 N2	5 N, 1 N2, 6 H	4 N	3 N, 5 H	36 N, 1 RN1	5 N1, 1 RN	1 RN1, 4 N 1 Rn2	1 RN1 1 N1	—
,, 6	—	—	—	2 N2, 1	2 RN2, 1	3 N	4 N	2 , 1	1 H, 4 RN2	5 N	1 RN1,	3 L
,, 14				1 RN2	12 H, 8 N1			1 RN2	2 N1		1 N2	
,, 14	—	—	—	1 , 2 ,	20 H, 1 N2 2	7 H	3 N2	2 , 2	3 H, 2 , 2 ,	3 , 2 H	1 , 1	1 N, 1 RN1
,, 20	—	—	—	—	1 , 2 ,	—	—	—	—	—	—	2 RN2
,, 22	—	—	—	—	—	—	—	—	—	—	—	1 , 1 ,
	2 H	1 H	4 H	2 H	20 H	7 H	5 H	0 H	3 H	2 H	0 H	0 H

Total no. of eggs laid, 86; total no. of hypopi, 46.

L = larva, RL = resting larva, N1 = protonymph, N2 = deutonymph, H = hypopus.

Table II.

Effect of different humidities on Glycyphagus domesticus.

Date	R.H. 10 %	R.H. 20 %	R.H. 30 %	R.H. 40 %	R.H. 50 %	R.H. 60 %	R.H. 70 %	R.H. 80 %	R.H. 90 %
Nov. 27	—	—	—	—	—	—	—	—	—
,, 30	All dead 1 egg	All dead	All dead	16 dead 13 alive	12 dead 14 alive	12 dead 15 alive	All alive 1 H, 1 egg	All alive 2 eggs	2 hypopi. All alive and breeding
Dec. 2	—	—	—	All dead	All dead	8 alive	13 alive 3 eggs	All alive 5 eggs	
,, 5	—	—	—	—	—	All dead	2 larvae 12 alive	4 L, 10 eggs All alive	—

Experimental conditions. The mites were kept in small cells made of glass rings ½ in. in diameter cemented on to glass slices. The tops of the cells were covered by cover-slips held in position by elastic bands. About five mites were placed in each cell, about thirty in all at each humidity. The humidity was kept constant by means of solutions of sulphuric acid in water in desiccators. The same experiment was repeated, using large numbers of mites in 2 in. by 1 in. tubes covered with silk. Rapid breeding occurred only in the tubes kept at a relative humidity of 80 and 90 per cent. The numbers in the tube kept at a relative humidity of 70 per cent. remained about constant.

Green Algerian fibre is a particularly suitable habitat for the mite. When placed in a damp atmosphere, it rapidly absorbs water and becomes covered with mould growth—mainly species of *Penicillium*. Horsehair is also fairly hygroscopic, but its horny casing makes it more resistant to fungal attack.

EFFECT OF HUMIDITY.

The Tyroglyphidae possess no specialised respiratory system and breathing takes place through the weakly chitinised body wall. Gaseous exchange can only take place through a moist limiting membrane, hence it is obvious that humidity must have a large controlling influence in the life of the mite. Indeed, the furniture mite is only found in large numbers where the rainfall of a locality is sufficiently great to provide a constantly high humidity. Table II shows the effect of different humidities on the increase in number of mites. It will be seen that the mites soon die at relative humidities below 60 per cent., but that at 70 per cent. the numbers remain fairly constant, and at humidities above 70 per cent. the mites reproduce rapidly. Hypopi are formed at the higher humidities.

The hypopial stage is extremely resistant to dryness on account of the low rate of metabolism associated with the simplicity of its structure. It can be kept over calcium chloride (relative humidity 10 per cent.) for a week and, when removed to a more favourable atmosphere, deutonymphs will start to emerge a few days later. About 40 per cent. of the total number of eggs laid pass through the hypopial stage. Hence to rid furniture or a room of mite attack by drying, the treatment must be repeated at intervals in order to kill off the deutonymphs as they emerge from the hypopial form.

Table III shows the effect of different humidities on the eggs. The eggs were exposed to different humidities for 1, 24 and 48 hours and then transferred to a relative humidity of 90 per cent. The experiment was not continued above 70 per cent. as the larvae started to emerge at that humidity. From this table it can be seen that the eggs are more resistant to low temperatures than the adults, that an exposure of 48 hours to a relative humidity of 10 per cent. is practically lethal, and that the number of larvae emerging is correlated with the relative humidity, more eggs hatching at higher humidities than at lower.

It was noted that the eggs which survived at low humidities were those in which development had only just commenced. Eggs which were due to hatch within a day collapsed when exposed to dry conditions.

Table III.

Effect of different humidities on hatching of eggs of Glycyphagus domesticus.

Time of exposure (hours)	R.H. 10 %			R.H. 20 %			R.H. 30 %			R.H. 40 %			R.H. 50 %			R.H. 60 %			R.H. 70 %		
	1	24	48	1	24	48	1	24	48	1	24	48	1	24	48	1	24	48	1	24	48
Time of hatching (days)																					
1	9L	1	—	—	—	—	1	—	—	—	2	—	—	1	—	—	1	—	—	2	9
2	3	—	—	—	—	—	1	—	1	3	2	—	2	2	—	12	—	—	20	—	—
3	—	—	1	—	1	—	10	1	—	10	5	—	24	6	—	—	—	18	12	6	6
4	—	3	—	19	23	—	20	—	—	19	—	—	—	—	35	—	34	13	10	29	31
5	25	13	—	16	14	—	—	7	6	—	—	36	—	10	3	33	1	—	—	2	—
6	4	9	—	5	5	—	—	9	6	—	31	1	14	20	—	—	—	5	—	—	—
7	—	5	—	4	2	3	10	10	—	11	—	—	1	—	1	—	1	—	—	—	—
8	—	—	3	2	—	6	—	—	9	1	—	1	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
10	—	—	—	—	—	9	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—
11	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total no. larvae emerged	41	31	4	47	45	18	32	27	22	45	41	38	42	39	39	45	37	36	42	39	46

No. of eggs used each time, 50. L = larvae.

Table IV.

Effect of temperature on emergence from hypopus of Glycyphagus domesticus.

Date	Temp. °C.	Dry 1 hr.	Sat. 1 hr.	Dry 24 hr.	Sat. 24 hr.	Con-trol	Temp. °C.	Dry 1 hr.	Sat. 1 hr.	Dry 24 hr.	Sat. 24 hr.	Temp. °C.	Dry 1 hr.	Sat. 1 hr.	Dry 24 hr.	Sat. 24 hr.	Con-trol
May 12	30	50 H	50 H	50 H	50 H	50 H	—	—	—	—	—	—	—	—	—	—	—
„ 17		2	1	—	—	8	—	—	—	—	—	—	—	—	—	—	—
„ 19		8	14	6	4	21	—	—	—	—	—	—	—	—	—	—	—
„ 20		6	5	17	7	4	—	—	—	—	—	—	—	—	—	—	—
„ 23		4	12	5	9	5	38	50 H	50 H	50 H	50 H		—	—	—	—	—
„ 24		—	—	—	—	—		—	—	—	—	42	50 H	50 H	50 H	50 H	50 H
„ 27		1	2	—	1	—		—	—	—	—		—	—	—	—	14
June 1		1	1	—	1	—		1	2	—	—		—	—	—	—	10
„ 3		1	1	—	—	1		9	14	—	—		—	—	—	—	6
„ 6		1	1	—	—	1		6	11	—	—		13	—	—	—	1
„ 9		—	1	—	—	—		3	—	1	—		11	—	—	—	1
„ 10		—	—	—	—	—		3	—	1	—		5	—	—	—	—
„ 13		—	1	—	2	—		2	—	4	1		5	—	—	—	2
„ 15		—	1	—	—	—		1	—	2	1		—	1	—	—	—
„ 23		1	—	1	2	—		—	—	2	—		—	2	—	—	—
„ 27		—	—	—	—	—		3	—	2	—		—	2	—	—	—
July 19		—	—	—	—	1		—	—	—	—		—	—	—	—	1
Sept. 20		—	1	—	—	—		1	—	—	—		—	—	—	—	1
Total no. of N2 emerged		25	41	29	26	41		29	27	12	2		34	5	0	0	36

Fifty hypopi were placed at the temperatures indicated for 1 hour and for 24 hours respectively under dry and saturated conditions. The numbers of deutonymphs that emerged are shown.

EFFECT OF TEMPERATURE.

A temperature of 23–25° C. is the optimum for the rearing of mite cultures. At this temperature the greatest number of eggs are laid and the life history only takes three weeks. At temperatures below 23° C., the life cycle may take from five to six weeks and a smaller number of eggs are laid by the females.

The effect of high temperatures on eggs and the hypopial stage is shown in Table IV and V. It can be seen from the figures that a temperature of 40° C. for 24 hours is lethal to both eggs and hypopi whether under dry or moist conditions, and that at temperatures above 38° C. fewer hypopi survive under moist than under dry conditions.

Table V.

Effect of heat on hatching of eggs of Glycyphagus domesticus.

Date (June)	Temp. (° C.)	Dry (eggs)	Wet (eggs)	Control (eggs)	Temp. (° C.)	Dry (eggs)	Wet (eggs)	Control (eggs)	Temp. (° C.)	Dry (eggs)	Wet (eggs)
1	—	—	—	—	37	50	50	50	—	—	—
3	32	50	50	50		10	6	23	41	50	50
7		—	—	16		10	9	13		—	—
9		11	9	12		3	13	—		—	—
10		2	4	8		4	7	3		—	—
13		18	18	11		—	2	2		—	—
14		—	1	—		2	—	1		—	—
15		1	1	—		—	—	—		—	—
Total no. of larvae emerged		32	33	47		29	37	42		0	0

Time of exposure: 24 hours.

The fact that temperatures above 40° C. are lethal to eggs, hypopi and adults could be used by the furniture trade as a means of sterilising all packing and upholstering materials before they were actually used in the manufacture and transport of furniture. Not only would this mean that warehouses would be kept free from infection but furniture could be guaranteed free from mite when purchased.

EFFECT OF CHEMICALS ON MITES.

All resting stages of *Glycyphagus domesticus* are formed in cracks, not only inside the furniture stuffing, but also in the floor and on walls and woodwork. Many of these mites can be killed if the room is consistently cleaned with a suitable disinfectant. Actual washing of floor and woodwork is more effective (and, incidentally, much cheaper) than spraying with insecticides. Soapy water and 5 per cent. formalin will not kill the active stages of the mite: they have even been kept alive in 5 per cent. formalin for several days. A 5 per cent. solution of carbolic acid will kill all stages, particularly if the solution is heated. Paraffin sprays are useless unless the mite is completely covered by the liquid: this is difficult because the droplets of paraffin are prevented from coating the mite by the long feathered hairs. The only methods of killing mites inside upholstered furniture are by continued and prolonged drying, by heating, which may spoil leather or fabric, or by gas fumigation.

Some experiments were made on the action of carbon tetrachloride and methyl salicylate on the hypopial form, details of which are given in Table VI. The figures indicate that methyl salicylate is very toxic at small concentrations, and that carbon tetrachloride is lethal if used in fairly strong doses.

Table VI.

Effect of carbon tetrachloride and methyl salicylate on hypopi of Glycyphagus domesticus.

A. Fumigant: carbon tetrachloride. Relative humidity: 80 %. Temperature: 20° C.
Duration of experiment: 18 hours. Feb. 14th. Number of hypopi used 50.

Tube	Dose	Result
1	0·1 c.c. per litre	13 deutonymphs emerged
2	0·2 ,,	5 ,,
3	0·3 ,,	0 ,,
4	0·4 ,,	0 ,,
5	0·5 ,,	0 ,,

Control nymphs. Forty-four emerged from fifty hypopi.

B. Fumigant: carbon tetrachloride. Relative humidity: 76 %. Temperature: 20° C.
Duration of experiment: 25 hours. Feb. 17th. Number of hypopi used: 50.

Tube	Dose	Result
1	0·1 c.c. per litre	None emerged
2	0·2 ,,	,,
3	0·3 ,,	,,
4	0·4 ,,	,,

Control nymphs. Forty-four emerged from fifty hypopi.

C. Fumigant: methyl salicylate. Relative humidity: 76 %. Temperature: 20° C.
Duration of experiment: 25 hours. Number of hypopi used: 50.

Tube	Dose	Result
1	0·02 c.c. per litre	None emerged
2	0·04 ,,	,,
3	0·07 ,,	,,
4	0·10 ,,	,,

Control nymphs. Forty-two emerged from fifty hypopi.

TYPES OF FURNITURE STUFFING.

The various materials used for furniture stuffing differ in their liability to become infested with mites.

Horsehair only harbours mites when it has not been properly cleaned.

Coir fibre is practically immune to mite attack. It is manufactured from the thick outer husk of the fruit of the coconut—*Cocos nucifera*.

Kapok is made from the seed capsules of *Eriodendron anfractuosum* and occasionally harbours *Tyroglyphus longior*.

Green Algerian fibre. All the bad attacks of mite observed have been associated with this fibre. It is made from the shredded leaves of the dwarf palm *Chamaeropes humilis* which grows wild in Algeria and Morocco. The fibre is twisted tightly into ropes in order to acquire a "curl" and is subsequently dried. The fibre is treated in various ways to render it more resistant to fungal and mite attack. Unfortunately, chemical treatment tends to make the fibre lose its resiliency and hence lessens its value as an upholstery material.

(1) It may be boiled in copper sulphate solution. Fibre treated in this way often harbours a considerable number of mites.

(2) It may be treated with copper sulphate and subsequently dyed with a wood dye akin to haematoxylin. Such fibre is immune from mite attack; it is, however, the most expensive type of fibre on the market and is less resilient than untreated fibre.

(3) The poorer grades of fibre are usually boiled with ferrous sulphate solution. This fibre is more resistant than the copper sulphate treated fibre and it seems likely that some modification of this treatment might place a perfectly reliable type of fibre on the market.

SUMMARY.

1. A list is given of the mites most commonly found in houses. The most important of these is *Glycyphagus domesticus*, the furniture mite, and this has been mainly studied during this investigation.

2. An account is given of the occurrence of *G. domesticus* in houses and the conditions under which it is found.

3. The life history is described. It is characterised by the occurrence of the hypopus, a cyst-like stage which is found only amongst the Tyroglyphidae.

5. Humidity and temperature have a marked effect on the life of the mite. Tables are given showing the effect of (*a*) humidity on the adult, (*b*) humidity and temperature on the hypopus and egg.

6. Some experiments on the effect of carbon tetrachloride and methyl salicylate on the hypopus are recorded.

This work has been carried out at the request of the High Wycombe and District Furniture Manufacturers' Federation under the direction of Prof. Munro of the Entomology Department of the Imperial College of Science to whom I wish to express my grateful thanks for all his help and encouragement. My thanks are also due to Dr Calman and Dr Finnegan of the British Museum (Natural History) for allowing me to make use of the facilities afforded by the Museum.

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OBSERVATIONS ON THE BIOLOGY OF THE *LYCTUS* POWDER-POST BEETLES, WITH SPECIAL REFERENCE TO OVIPOSITION AND THE EGG

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(With 9 Text-figures.)

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INTRODUCTORY.

POWDER-POST beetles of the family Lyctidae are cosmopolitan in distribution, and a number of the species which are found attacking commercial hardwood timbers are of great importance. A recent survey of the present economic position of these beetles with reference to many branches of the hardwood-using industries in Great Britain is given by Fisher, Cann and Parkin (13). The sapwood of certain recently seasoned hardwoods with large vessels, *e.g.* oak, ash, elm, sweet chestnut, walnut, hickory, etc., is very liable to severe damage. The *Lyctus* larvae tunnel through the wood parallel to the long axes of the vessels and, in time, may reduce the wood almost completely to a fine, flour-like powder: a thin outer skin, however, is left intact, but for the numerous exit holes of several generations of beetles.

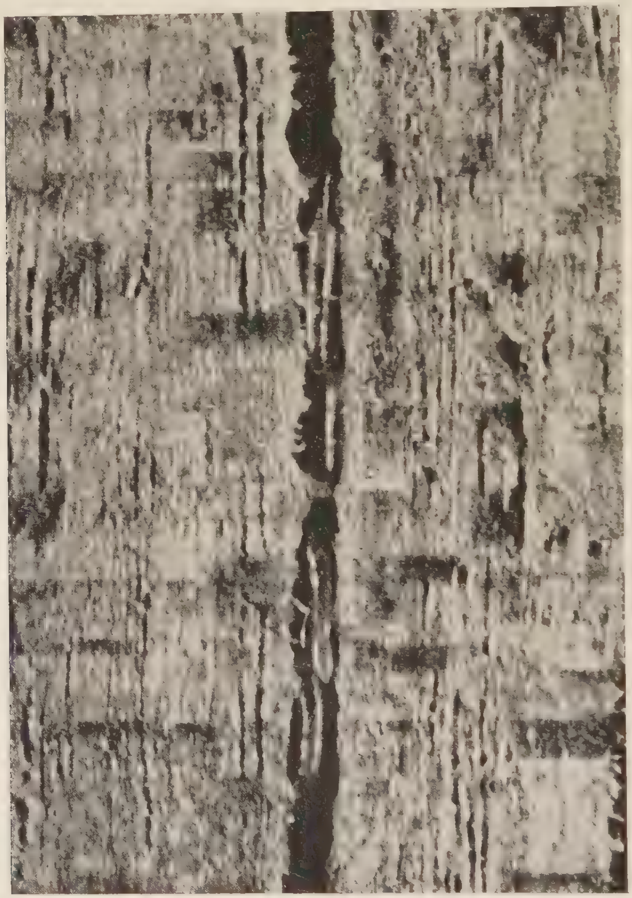

Fig. 1. Eggs of *L. brunneus* in vessel of English oak. (× 18 approx.)

An investigation into the biology and methods for control of the powder-post beetles has been in progress at the Forest Products Research Laboratory, Princes Risborough, since 1925, and an account of the work done up to 1928 has been published by Fisher (12). In the course of this work, Fisher (11, 12) and Clarke (9, 10) investigated some of the factors influencing attack by *Lyctus* in timber and showed that there was a relation between the diameter of the vessels of a wood and its liability to attack. This "pore-size" relationship depends on the fact that *Lyctus* beetles lay their eggs within the pores or vessels (Fig. 1) and, therefore, timbers of which all the vessels have a diameter less than the diameter of a *Lyctus* egg should not be liable to infestation as oviposition cannot take place. The relationship between vessel size, diameter of egg and liability to attack is well shown graphically in both of Clarke's papers. This work was based upon measurements of vessel diameter for a large number of woods which were known from laboratory records and experiments either to be liable to infestation or to be apparently immune. There were, however, several records (1, 19) in the literature on the Lyctidae of infestation in woods which, according to the theory formulated, should not have been liable to attack. Some of these records seemed doubtful, but a few evidently formed exceptions to the pore-size theory, and no explanation was attempted at the time. Experiments were started, however, to ascertain whether eggs could be laid in small-pored timbers, and examination of the samples showed the presence of eggs in several timbers in which oviposition was not expected to take place. Moreover, about the same time, a plank of English sycamore attacked by *Lyctus brunneus* was received at the Forest Products Research Laboratory; sycamore wood is a small-pored timber and should not have been liable to attack.

Having determined that *Lyctus* beetles could lay eggs in certain small-pored timbers, a detailed investigation was started to discover how these exceptions to the pore-size relationship could be accounted for and the results of this investigation are embodied in the present paper. Information on the biology of various species of *Lyctus*, which is additional to that already published, has also been included since the observations were made during this special study of the egg and method of oviposition.

Nothing need be said of the synonymy of the species dealt with here, except that *L. canaliculatus* F. and *L. linearis* Goeze are synonyms.

LITERATURE AND PREVIOUS WORK.

In 1853, Heeger (14) described the egg of "*Lyctus pubescens*," but all recent workers are agreed that the beetle in question did not belong to the family Lyctidae. Nördlinger (17), whose paper I have unfortunately not been able to obtain, stated, according to other investigators who have quoted his work, that the eggs of *L. linearis* are laid in cracks and fissures in the wood. Xambeau (24) gives an account of the emergence, mating and oviposition of *L. canaliculatus* and describes briefly the egg: Bureau (5) repeats Xambeau's statements. More recently a considerable amount of work has been done upon the biology and habits of the *Lyctus* powder-post beetles, particularly by Snyder (21) in the United States of America, Altson (1, 2, 3, 4) and Fisher (11, 12) in Great Britain, and Kojima (15) in Germany. Chowdhury (7) has recently published the results of his investigations into the liability of Indian timbers to *Lyctus* attack based upon Fisher and Clarke's pore-size relationship.

MATERIAL.

The investigation detailed in this paper was carried out primarily upon *Lyctus brunneus* Steph., now the common species in Great Britain, but a considerable amount of work was also done with *L. linearis* Goeze, which occurs in British timbers, and with *L. planicollis* Le C., a species frequently found in timber imported into Great Britain from U.S.A. A few additional observations are recorded upon *L. parallelopipedus* Melsh., and *L. cavicollis* Le C. (18), both North American species, and *L. sinensis* Lesne (18) which was taken from imported Japanese oak. All the beetles were collected from infested timber stored in insectaries at the Forest Products Research Laboratory. Specimens were identified at the laboratory and representative insects were submitted for confirmation to Mr W. S. Fisher, U.S. National Museum, Washington, D.C., and to Mons. P. Lesne, Muséum National d'Histoire Naturelle, Paris, to whom thanks are due for their willing help.

LIFE HISTORY AND HABITS OF *LYCTUS*.

The eggs of the *Lyctus* powder-post beetles usually hatch in 8–12 days and the young larvae commence tunnelling in the wood, where they feed and grow until reaching maturity some ten months later. The fully grown larva approaches the surface of the wood, constructs a pupal cell just below it and then pupates. After 3 weeks or more the beetle sheds the pupal skin, but remains within the wood for another 3–4 days to harden the chitin before eating its way to the outside through a small round exit hole about 2 mm. in diameter. After emergence from the wood the beetles pair and the females start egg-laying, thus completing the life cycle. In Great Britain there is one generation per year, the insect hibernating as a half-grown or nearly fully grown larva. Under laboratory conditions beetles can be obtained all the year round, though they are usually scarce during the winter months. In the open, the main emergence takes place during June, July and August, but a few beetles may be found during May and September if the weather is favourable. Altson (4) states that the females considerably outnumber the males and that one male must therefore fertilise several females. This is not substantiated by the emergence records for *L. brunneus* kept at the Forest Products Research Laboratory during the past five years, which show an emergence of 3193 males to 2293 females, or a sex ratio of 7 : 5 males to females.

The length of life of the adult insects seems to vary considerably, but is usually 3–6 weeks. Some beetles die within a few days of emergence, and the maximum period so far recorded was attained by a female *L. brunneus* which survived 68 days; the longest span of life for a male of the same species was 54 days. On the whole, there was little difference in length of survival between the two sexes. The maximum periods for length of adult life of *L. brunneus* are very much greater than those found by Kojima (15) for *L. linearis*, who gives the maximum duration of life at room temperature for this species as 23 days for females and 32 days for males. It is not known how long the female is engaged in oviposition, but a beetle has been found ovipositing 40 days after the commencement of an experiment, and from information gained in dissections it would seem that the beetle continues to live for several days at least after ceasing oviposition.

The activity and reactions of the beetles are closely related to the conditions of temperature, light, etc., prevailing. The beetles become very active at temperatures above 20° C., when they run about on the surface of the wood, but such activity is not usually seen during the daytime. Throughout the day, the insects usually crawl into the small spaces between boards, etc., or go into old exit holes, but as soon as it is dusk they become very active; they readily take flight and are quite strong fliers.

SECONDARY SEX CHARACTERS.

The secondary sex characters of these beetles have not been fully described, and an account is given here of the sexual differences for six species of *Lyctus*, as the presence of external characters by which the sexes may be distinguished saves much needless dissection. In general, these characters are but poorly developed in the Lyctidae except in *L. brunneus*. As Altson (4) points out, size is no guide to sex. For species other than *L. brunneus*, the differences between the sexes show best in the middle half of the posterior margin of the mentum. Detection of the males is often rendered easy by the adherence of small particles of wood to the long erect hairs on the mentum which thereby give the beetles the appearance of possessing a whitish "beard." *L. brunneus* is exceptional since it does not show a secondary sex character on the mentum, as do all the other species examined. However, another character, given below, is very easy to observe.

Lyctus brunneus Steph.

Altson (4) has described a secondary sex character for this species, but neither his description nor his figure seem very clear. The following definite character has, however, been utilised at the Forest Products Research Laboratory for several years:

Female. Near the posterior margin of the last visible abdominal sternite is a small but distinct median "pencil" of yellow setae.

Male. The pencil of setae is lacking.

Lyctus linearis Goeze.

Male. The mentum is furnished with a marginal series of relatively long, fine, erect, yellowish grey setae, all of which are curved towards the centre of the mentum. There is also a similar small group on the submentum. The length of the setae is about equal to the length of the mentum.

Female. On the mentum and submentum is a series of short, fine, decumbent yellow setae the length of which is scarcely one-half the length of the mentum: one or two longer erect yellow hairs may occur just in front of the posterior angles of the mentum.

Lyctus planicollis Le C.

As Lesne (16) points out, the secondary sex characters of this species are very feeble.

Male. The length of the fine erect anteriorly recurved setae on the posterior margin of the mentum nearly equals that of the mentum.

Female. The mentum is sparsely covered with short, fine, decumbent setae, the length of which is only about one-sixth that of the mentum.

Lyctus parallelopipedus Melsh.

Male. On the posterior and lateral margins of the mentum there is a row of very long, fine, erect, anteriorly recurved yellow setae; the length of these setae, which are particularly noticeable at the posterior angles, is about twice the length of the mentum.

Female. There are no setae as described above on the posterior margin or posterior angles of the mentum, but there is a series of shorter setae on the lateral margins: these setae are about equal in length to the length of the mentum.

Lyctus cavicollis Le C.

Male. On the mentum is present a group of fine, erect yellowish grey setae all curved towards the centre of the mentum; the setae are about three-quarters the length of the mentum. Shorter, erect setae are present also along the anterior border of the submentum.

Female. There is a group of decumbent yellow setae on each side of the mentum, but they do not extend along the whole of the posterior margin; the length of the setae equals about one-half that of the mentum. On the submentum there are only a few scattered, short decumbent setae.

Lyctus sinensis Lesne (Fig. 2).

Male. The mentum is covered with moderately short, fine, erect yellowish grey setae which are recurved towards the centre of this sclerite; the length of the setae is little more than one-half the length of the mentum. Similar setae are present on the submentum.

Female. The mentum is furnished with very short, decumbent yellow setae not exceeding in length one-third that of the mentum. The submentum is almost devoid of setae.

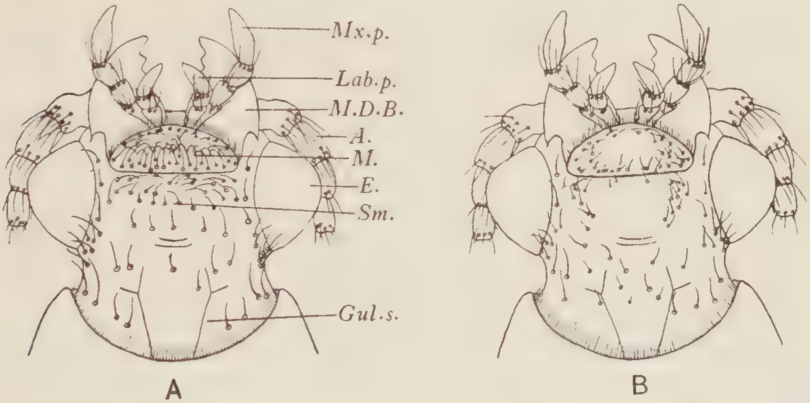

Fig. 2. *L. sinensis.* Ventral view of head. A, male; B, female. *A*, antenna; *E*, eye; *Gul.s.* gular suture; *Lab.p.* labial palp; *M*, mentum; *M.D.B*, mandible; *Mx.p.* maxillary palp; *Sm.* submentum.

MALE REPRODUCTIVE ORGANS.

The male reproductive organs of *L. brunneus* have been described by Altson (4), and those of *L. linearis* by Kojima (15), and only brief mention will therefore be made in this account of the various parts of the genital system. The testicular follicles consist of two groups of fairly large pear-shaped bodies connected by short vasa efferentia to a long convoluted vas deferens on each side. Altson found 6 follicles in each testis of *L. brunneus*. Kojima states that 7 is the number for *L. linearis*, but the writer has not found more than 6 follicles per testis for either *L. brunneus* or *L. linearis*; some specimens seem to have only 5, or 5 on one side and 6 on the other. When all the spermatozoa have been discharged, the follicles shrink greatly in size until they appear as no more than small terminal dilations of the vasa efferentia. Each vas deferens opens into a large vesicula seminalis to which is closely attached posteriorly an equally large colleterial gland. The vesiculae are held close together by the short ducts leading into the single ductus ejaculatorius which runs posteriorly into the well chitinised aedeagophore.

The spermatozoa of *L. brunneus* are long, extremely slender, motile bodies, slightly but not very obviously thickened at one end, presumably the anterior or "head" end.

When examined in a stained smear, nearly all are seen to be flexed like a *Spirillum*. Dissection of beetles in 0·75 per cent. salt solution shows that the spermatozoa are motile. The total length is about 66 μ, of which the "head" makes up about 15 μ; the diameter of the head end is approximately 0·9 μ.

The male copulatory apparatus, or aedeagophore, of *L. linearis* has been described by Sharp and Muir (20) and Kojima (15), and that of *L. brunneus* by Altson (4). The aedeagophores of several species of *Lyctus* have been examined, but the differences between them are very slight and for the purposes of this paper are too small to merit description.

FEMALE REPRODUCTIVE ORGANS.

The female reproductive organs of *L. brunneus* and *linearis* have been described by Altson (4) and Kojima (15) respectively: these organs are very similar in the two species and also, as the present work has shown, in *L. planicollis*, *L. parallelopipedus* and *L. sinensis*.

Each ovary consists of a number of ovarioles of acrotrophic type, which is the form usually occurring among the Coleoptera-Polyphaga. These ovarioles number 14 per ovary in *L. brunneus* according to Altson, and Kojima gives 7 ovarioles per ovary in *L. linearis*. From numerous dissections made during the course of the present investigation, it would seem that in *Lyctus brunneus* the number of ovarioles is variable, 12 being the number found most frequently but from 10 to 16 occurring quite commonly; there may even be a different number of ovarioles in the two ovaries of the same female. In *L. linearis* 7 ovarioles per ovary is the rule, though 6 only may sometimes be found. *L. sinensis* resembles *linearis* in having 7, whilst in *L. planicollis* there are 6 and in *L. parallelopipedus* apparently 8 ovarioles to each ovary.

Usually, one mature or nearly mature egg can be seen in a number of the ovarioles and there are, in addition, the primordia of 2–3 additional eggs. When the eggs are mature, they pass out of the ovariole into the calyx, which is a greatly expanded distal portion of the oviduct. The calyx is thin-walled and in it the eggs are stored before being laid. Eggs pass into the calyx before the female is fertilised, so that she is presumably ready to begin oviposition very soon after fertilisation. It is stated by Altson (3) that, if the female remains unfertilised, eggs continue to pass into the calyces thereby causing great distension, and finally bringing about the death of the beetle. In the present work it has been confirmed that the calyces become distended with eggs, but no experiments were made to find out whether unfertilised females died sooner than those which had mated. In *L. brunneus* it is quite common to find recently emerged but fertilised females with a total of 30–40 eggs in the calyces; the maximum recorded was 50. There are usually approximately equal numbers of eggs in both calyces whether the total number be large or small. *L. linearis* seems to have considerably fewer eggs stored in the calyces than *L. brunneus*, the total number being commonly only 4 or 5; the maximum number found was 23, but in this case the female had not been fertilised. In *L. planicollis*, any number between 5 and 25 may occur, but in one instance the calyces of a fertilised female of this species contained the record number of 56 eggs. The number of eggs occurring in the calyces is significant in connection with determination of the number of eggs laid by one beetle; this will be discussed later.

Altson (4) found that the left ovary of *L. brunneus* always matured earlier than the right, but this could not be confirmed. So far as could be seen, both ovaries developed and matured simultaneously, and this is supported by finding approximately equal numbers of eggs in right and left calyces. The right and left calyces are continued posteriorly as the corresponding oviducts, which are thick-walled and muscular, and unite to form the long, slender muscular common oviduct which leads into the ovipositor.

At the point of junction of the right and left oviducts there arises a large sac-like spermatheca to which is attached apically a long, slender spermathecal gland, the secretion of which, as Kojima suggests, may serve to liberate the sperm from the bundles in which they are introduced and also to sustain them. In fertilised females the spermatheca is cream coloured and distended with spermatozoa. There is no bursa copulatrix present, the spermatozoa passing straight up the common oviduct into the spermatheca. In a female which was dissected three hours after mating it was found that all but a very small quantity of the sperm had already passed into the spermatheca. In *L. parallelopipedus* there seems to be an additional slender duct running from the apex or upper half of the spermatheca to the common oviduct into which it opens at a point just opposite the fusion of the paired oviducts. It is not known what the function of this duct can be; it was not discovered in any of the other four species dissected. Unfortunately, only two females of *L. parallelopipedus* were available for examination but this curious connection between common oviduct and spermatheca was quite clear in both specimens.

The right and left oviducts are flattened dorso-ventrally immediately in front of their point of fusion and are here provided with a well chitinised lining upon which are set numerous long, backwardly directed chitinous bristles. These chitinised flattened portions of the oviducts have been called "valves" by Altson (3, 4), who considers that by their action the eggs acquire the process and striations at the cephalic pole which will be described later. Kojima (15) agrees with Altson, but if this is correct, it is difficult to see why the whole of the egg should not be striated when passing the valves. Three other possibilities may be considered: firstly, the valves may prevent spermatozoa entering the two oviducts during copulation: secondly, passage of eggs through the valves may in some way cause spermatozoa to be liberated from the spermatheca for fertilisation of the eggs; thirdly, the valves may resist the passage of the egg and cause it to be squeezed in order to get through, thus resulting in a reduction in the diameter of the egg with a corresponding increase in length. This last view is of importance in connection with egg-laying and will be dealt with again when considering the passage of the egg from calyx to wood vessel during oviposition.

The ovipositor is a long, slender tube with a chitinised inner wall and a colourless transparent outer sheath. It is normally completely retracted within the body but can be everted in the form of a single tube, when its length may exceed that of the body. When extruded, the chitinised inner wall forms the distal portion and the colourless outer sheath becomes the proximal part. The ovipositor terminates in a pair of small two-segmented palps between which is the female genital orifice.

Copulation and oviposition.

Copulation, as previously stated by Altson (3, 4), appears to take place almost immediately after emergence. The beetles pair chiefly in the evening but may also mate during the day if the weather is warm. Judging from observations on the living beetles and from dissections of males and females at various periods after emergence, both sexes may mate more than once.

In the female, the spermatheca is very large and is often greatly distended with spermatozoa. In females which have definitely paired only once the spermatheca is usually only half full, so that these beetles possibly mate two or three times. It has also been noticed that females which have their ovaries nearly exhausted are occasionally found with the spermatheca quite full.

Oviposition takes place mainly at the period of greatest activity, *i.e.* at dusk, but may also occur during the day. As mentioned before, the eggs are laid within the open pores or wood vessels, the female inserting the ovipositor into the pore so that the egg is laid some distance from the surface (Fig. 1). Vessels opening on either the transverse or longitudinal surfaces of the wood are utilised, and in timbers having a large range of vessel size, the pores chosen are usually not the largest but those with a diameter only slightly greater than the "normal diameter" of the egg. The term "normal diameter" is used to denote the diameter assumed by the egg after being laid in a place where it is not compressed or restricted in any way. Nördlinger (17) and Xambeau (24) state that the eggs of *L. linearis* are laid in cracks and crevices in wood, but this statement has been disproved by Fisher (12) who showed that the eggs of this species were laid within the vessels like those of *L. brunneus* (Altson (3)) and *L. planicollis* (Snyder (21)). In the seven years during which work on the *Lyctus* powder-post beetles has been carried out at the Forest Products Research Laboratory, only two instances have been observed in which eggs were laid in cracks. Both were in the same series of experiments in which samples of birch were exposed to attack by *L. brunneus* and eggs were found in small cracks in the pieces; these eggs hatched normally and the young larvae started to bore in the wood. Since birch is a small-pored timber and the beetles could lay their eggs only on the pieces provided, it is not unlikely that the beetles used the cracks from necessity rather than choice. There can be little doubt that oviposition within the pores of suitable timbers is the normal method among the *Lyctus* powder-post beetles.

It has always been stated that coniferous timbers are not liable to attack since vessels are not present in these woods. The tunnels of young larvae have, however, been noted in a small block of Scots pine exposed to attack by *L. brunneus* and, although the larvae had died before tunnelling very far, it was obvious that eggs had somehow been laid in the sample, which did not show any signs of cracks or crevices. Careful examination showed that the eggs had been laid in the empty portions of resin canals, which are quite wide tubes comparable in diameter with the vessels of many hardwoods.

The number of eggs laid in a single pore varies from 1 to 8. The number most frequently found in a pore also varies according to the species of *Lyctus* concerned. Data collected on this question for the two species, *L. brunneus* and *L. linearis*, are set out in Table I.

Table I.

Frequency of occurrence of a given number of Lyctus *eggs in a vessel.*

	Number of eggs per vessel							
Species	1	2	3	4	5	6	7	8
L. brunneus	29	42	60	28	13	5	—	1
L. linearis	121	98	45	14	1	—	—	—

These results show clearly a difference in the habits of the two species with reference to egg-laying. *L. brunneus* lays most frequently three eggs in a vessel, whereas *L. linearis* usually lays but one. 508 eggs of *L. brunneus* and 513 of *L. linearis* were located during the course of this investigation.

A further difference between the two species can be seen in the maximum number of eggs found in a vessel. Whilst *L. linearis* laid 5 eggs in a pore only once and a greater number was never found, *L. brunneus* not infrequently laid 5 in a pore, five pores were found containing 6 eggs and on one occasion 8 eggs were discovered in a single vessel. It is almost certain that all the eggs in a pore, provided that their anterior ends all point in the same direction, have been laid by one female since the eggs are always placed in a single row and do not appear to slide about in the vessel; the last egg laid is usually 2–3 mm. from the surface. If only one egg was laid in a pore, therefore, it would be necessary for a beetle wanting to lay additional eggs in this same pore to push the original egg backwards before inserting its own; as the eggs are very fragile they would probably be crumpled or broken in the process. In the case of the 8 eggs in one pore quoted above, the posterior end of the first egg laid was approximately 7·75 mm. from the surface of the wood on which the vessel opened: distances of from 6 to 7 mm. are quite common. It is probable that these 8 eggs were laid by a beetle with an exceptionally long ovipositor; the longest ovipositor actually measured was 6·4 mm., whilst the average length is about 5 mm.

With regard to the number of eggs laid by one female, Kojima (15) has found that an average of 15–20 is usual for *L. linearis*. As he points out, this figure seems very low, but his results have been confirmed at this laboratory in two experiments with *L. brunneus*, in which 12 females laid an average of 17·4 eggs, and 18 females 21·2 eggs per beetle respectively. The figures were obtained by counting the total number of exit holes made during the year in the samples of English oak sapwood attacked. It is therefore possible that more eggs per female were laid, some failing to hatch, or some of the larvae proving unable to complete their development. Assuming that a female *L. brunneus* has an average of 14 ovarioles per ovary (see p. 502) and that only 2–3 eggs mature from each ovariole, the total number of eggs producible by a female should be 56–84, or an average of 70: in *L. linearis* with only 7 ovarioles per ovary the average number of eggs producible by a female is reduced to 35. In the case of *L. planicollis* in which 56 eggs were found in the calyces and only 6 ovarioles per ovary, at least 4–5 eggs must have matured in each ovariole. The individual variation in the number of eggs laid must be very great since some beetles collected immediately after emergence from the wood failed to lay more than one or two eggs before dying, whilst in other cases dissection showed that nearly all the eggs had been laid.

Females dying after the completion of oviposition normally show, at the most, one or two malformed eggs in the calyces with an occasional half-mature egg in a few of the ovarioles. Some beetles, then, probably do lay a number of eggs which approaches the maximum possible, but the average number laid by a group of females will be much lower owing to a few beetles dying after depositing a very small number.

DESCRIPTION OF THE EGG.

The eggs of *Lyctus* spp. were obtained by enclosing a small sample of recently seasoned English oak sapwood in a glass tube or glass-topped tin with a number of newly emerged beetles. The samples were first prepared by cleaning the transverse surfaces of the wood with a chisel in order to open as many pores as possible. After two or three days the samples were examined by carefully shaving off thin sections under a binocular microscope. Eggs of *L. brunneus*, *linearis*, *planicollis* and *sinensis* were obtained for observation in this manner.

Lyctus eggs are unusual among those of Coleoptera owing to the curious process or strand at the anterior end which somewhat resembles that found in eggs of certain parasitic Hymenoptera. Among the Coleoptera the egg of *Lyctus* is most closely approached by that of certain members of the closely related family, Bostrychidae, as described by Burke, Hartman and Snyder (6) and by that of *Tarsostenus univittatus*, Rossi (St George (22)), a Clerid predator of *Lyctus*, which also lays its eggs in the wood vessels. *Lyctus* eggs are not attached to the wall of the vessel so that it is impossible to explain the strand as an anchoring process. The eggs of the various species of *Lyctus* examined resemble each other very closely.

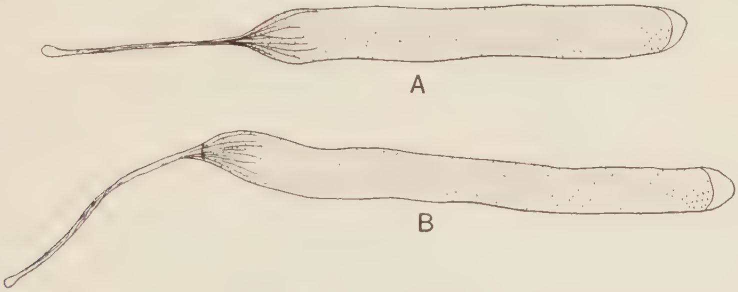

Fig. 3. *L. brunneus*. Eggs. A, normal egg; B, normal long egg.

The egg of *L. brunneus* (Fig. 3) has been briefly described by Altson (3). It is long, slender, cylindrical and does not show any appreciable dilation at the anterior end. The whole egg is translucent and white in colour with the exception of a short part posteriorly which is quite transparent, devoid of yolk and occupies about one-twentieth the length of the egg body. The yolk globules which fill the egg are slightly smaller in the posterior half than in the anterior half. The chorion is very thin and fragile, more so posteriorly than anteriorly, and is very shiny. Imposed on the chorion is a series of extremely fine longitudinal striae which are often visible anteriorly but gradually disappear about halfway along the egg. At the anterior or cephalic pole the chorion is continued in the form of a thin process or strand, terminating in a minute ampulla, devoid of contents and showing numerous longitudinal foldings or wrinkles, which are continued backwards over the tapering anterior part of the main egg body. The dimensions are given in Table II, and are based upon measurements of 34 eggs.

There is considerable variation in the dimensions of the eggs of *Lyctus* and this is probably connected with the great range of size of the beetles. Fisher (12) has published a series of measurements of 20 eggs of *L. brunneus* which show an average length of egg body of only 833 μ. A further series of 20 eggs examined by the writer gave an average length of 996 μ. It can therefore only be assumed that the size of the eggs depends upon the beetles used in the experiment; probably an average figure of about 1000 μ is correct.

Table II.

Dimensions of the egg of Lyctus brunneus *Steph.*

	Dimension		
	Average	Maximum	Minimum
Length of egg body	1164 μ	1360 μ	809 μ
Diameter of egg body	142 μ	180 μ	125 μ
Length of strand	418 μ	785 μ	215 μ
Diameter of strand	14 μ	26 μ	9 μ

The egg of *L. linearis* (Fig. 4 *a*) resembles that of *L. brunneus* in its general description and colour, but is shorter and wider and shows a well marked widening or dilation at the anterior end. No transversely wrinkled constriction was visible behind this bulbous part as mentioned by Kojima (15). The anterior end is less tapering than in *L. brunneus* and the strand is, in consequence, more clearly demarcated from the egg body. Posteriorly there is a very obvious transparent portion amounting to about one-tenth the total length of the egg body. The yolk globules are smaller than in *L. brunneus*, of equal size throughout and very tightly packed, so that the egg is rather more opaque. The chorion is shiny and wrinkled longitudinally on the strand and on the bulbous portion of the egg. The average dimensions of the egg are:

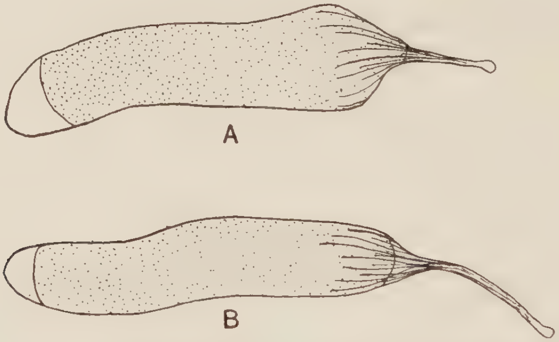

Fig. 4. Normal eggs. A, *L. linearis*; B, *L. planicollis*.

Length of egg body	817 μ
Diameter of egg body	154 μ
Diameter of bulbous anterior end	194 μ
Length of strand	211 μ
Diameter of strand	14 μ

A general description of the egg of *L. planicollis* is given in a short paper by Snyder (21). It is a rather short, thick egg (Fig. 4 *b*) but unlike that of *L. linearis* it has no appreciable bulbous anterior portion and the change from egg body to strand is not so sharply demarcated. The posterior end is clear and transparent. The yolk globules are somewhat smaller in the anterior half. The chorion is moderately shiny and marked all over with extremely fine longitudinal striations which under high magnification appear like shallow grooves set very closely together. Wrinkling of the chorion is visible over the anterior end of the egg and on the strand. The average dimensions of the egg of this species are:

Length of egg body	881 μ
Diameter of egg body	138 μ
Length of strand	273 μ
Diameter of strand	14 μ

The egg of *L. sinensis* resembles that of *L. linearis* in that it is fairly short and is definitely swollen at the anterior end, but there are practically no longitudinal wrinkles on the bulbous portion except near the point of transition into the relatively long strand. No markings are visible on the chorion apart from the wrinkles just referred to. The posterior transparent portion is moderately large, amounting to about one-fifteenth the length of the egg body; the yolk globules in the rest of the egg are about equal in size throughout. Only 5 eggs were obtained for measurement and the averages of the dimensions recorded are:

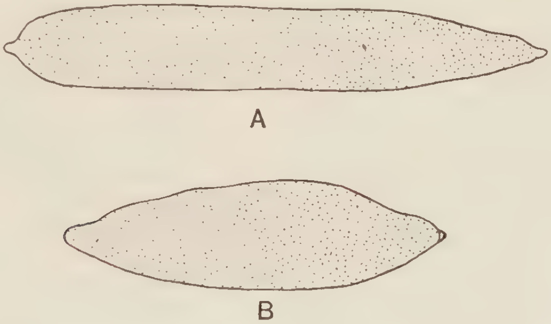

Fig. 5. Eggs removed from calyx. A, *L. brunneus*; B, *L. linearis*.

Length of egg body	743 μ
Diameter of egg body	131 μ
Diameter of bulbous anterior end	162 μ
Length of strand	466 μ
Diameter of strand	14 μ

The eggs from the calyces of all four species examined are very similar to each other, and, taking *L. brunneus* again as the type, it is immediately obvious that there are considerable differences between the eggs before and after being laid. When dissections were carried out in water, it was found that the eggs always adhered very strongly to one another by their anterior ends and it was impossible to separate them without tearing the very elastic chorion, or, at least, considerably distorting the eggs, but this difficulty was overcome by dissecting in 0·75 per cent. salt solution. Eggs removed from the calyx (Fig. 5 *a*) are fusiform, tapering rather more anteriorly than posteriorly; they lack any anterior bulbous portion and as pointed out by Altson (3) the strand and longitudinal wrinkling are absent. The difference between the egg in the calyx and the egg after being laid can be accounted for only by modification of the form of the egg during or immediately after oviposition.

The average dimensions of eggs from the calyx are as follows:

Species	Length	Diameter
L. brunneus	1071 μ	169 μ
L. linearis	725 μ	200 μ
L. planicollis	893 μ	187 μ
L. sinensis	801 μ	185 μ

The diameter of the egg in the calyx is considerably greater than the diameter of the laid egg.

Although there is no strand present on the egg before being laid, there is in *L. sinensis* a tubular prolongation of the anterior end of the egg (Fig. 6 *a*) which at first sight resembles a thick strand. Its diameter, however, is about four times and its length only one quarter that of the true strand and it shows none of the characteristic creases of the chorion. Furthermore, it is traversed internally by a protoplasmic process from the yolk mass, which suggests that it may play a part in fertilisation. No sign of a micropylar opening could be detected in the egg of any species but the anterior end is covered with a gelatinous cap (Fig. 6 *b*) which is no doubt connected with the process of fertilisation; it is probably these caps which make the eggs stick to one another when the beetles are dissected in water. Immediately below the cap is a layer of protoplasm which is separate from the main yolk mass and contains no yolk globules. There is a small peg-like projection at the posterior end of the egg as well but this is filled with yolk globules.

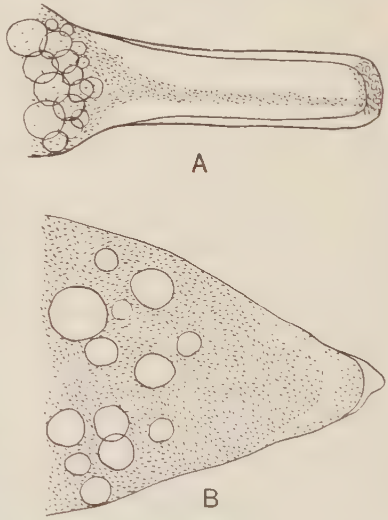

Fig. 6. Anterior end of egg from calyx. A, *L. sinensis*; B, *L. linearis*.

There are thus considerable differences between laid eggs and eggs in the calyx. How can these differences be accounted for? It is highly improbable that these changes take place after the laying of the egg, and attention may therefore be confined to the changes undergone during passage from the calyx through the oviduct and ovipositor to the exterior. On leaving the calyx the egg passes into one of the paired oviducts which are thick-walled, muscular and capable of considerable expansion. When, however, the egg has to pass through the narrow, flattened, setose opening of the valve its diameter must be markedly decreased and its length correspondingly increased. The egg is presumably forced through the valve by contraction of the muscular walls of the paired oviduct and the increase in length is allowed for by the great elasticity of the chorion. This elasticity is easily demonstrated by removing some eggs from a calyx under water and then attempting to separate them by pulling them apart: the choria will stretch surprisingly before tearing. It is in passing the valve that the egg probably becomes fertilised and the elongated egg then travels along the narrow but muscular common oviduct into the ovipositor. At the posterior end of the ovipositor a small part of the egg is squeezed to the exterior, *i.e.* into the cavity of a wood vessel, and the pressure exerted by the walls of the ovipositor causes some of the egg contents to be forced into the extruded part of the egg body, thus swelling it again up to its final diameter which is a little less than that of the egg in the calyx. The chorion, not being perfectly elastic, does not contract completely to its original length, so that a part is left over containing no yolk. This portion of the chorion collapses and thus forms the wrinkled strand, the creases extending posteriorly on to the egg-body to allow for a close fit of the chorion over the tapering anterior part of the egg.

The process of egg-laying described is necessarily suppositional. What evidence is there to support this view? In the first place, the egg must be compressed to pass through the narrow lumen of the chitinised valve. Secondly, unless the egg had been considerably increased in length, there seems to be no possible explanation of the origin or use of the strand, since, as stated before, the strand does not serve to attach the egg to the wall of the vessel. Thirdly, this supposition explains the occurrence of the longitudinal folding or wrinkling on the strand and over the anterior end of the egg. Furthermore, the ovipositor has an internal diameter smaller than that of the laid egg and, being chitinised, is presumably not capable of much expansion. Finally, one of the strongest pieces of supporting evidence is found in the occasional occurrence of imperfect or "freak" eggs. These eggs (Fig. 7) are always considerably longer than the normal eggs and in most cases clearly show the presence of yolk in part of what should have been strand. In one instance, a long slender irregularly shaped egg had no strand but yolk globules extended the full length of the chorion. More often, however, in these abnormal eggs, the egg body is elongated and is constricted somewhere in the anterior half so that a portion of the yolk is partially nipped off from the main mass and the strand is therefore shortened. Longitudinal wrinkling is usually visible on the constricted portions, showing that this wrinkling probably compensates for the variation in diameter of the imperfectly elastic chorion. Apart from these moderately rare abnormalities, there occur very frequently imperfections (Fig. 8) at the point where the egg tapers into the strand and all of these departures from the normal lend support to the view that the strand has at one time been filled by the yolk mass, which has later receded into the main egg body.

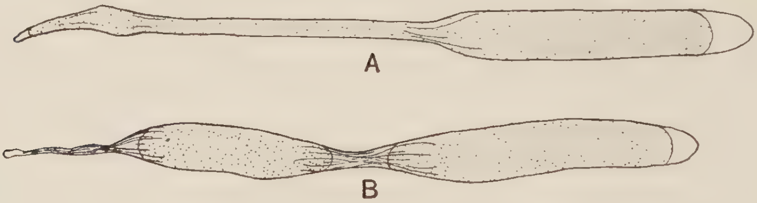

Fig. 7. *L. brunneus.* Abnormal or "freak" eggs. A, no strand present; B, showing subdivision of the yolk.

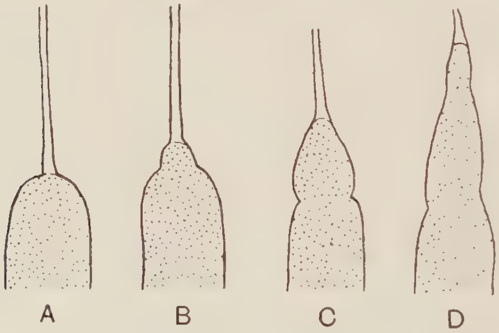

Fig. 8. *L. brunneus.* Differences in junction of egg body and strand.

It is interesting to note that Chrystal (8) has given a similar explanation of the passage of the egg from the ovary to the exterior in *Ibalia leucospoides*, a Cynipid parasite of the *Sirex* wood wasps, but in *Ibalia* a strand is present on the egg whilst in the ovary and it does not arise during the course of oviposition. He says that "the eggs of *Ibalia* are typical of the Cynipoidea, being milky white in colour and pedunculate.

As they lie in the ovary the sausage-shaped egg-body points towards the oviduct, which position is characteristic of all the Cynipoidea, and distinguishes them from some other parasitic Hymenoptera, such as *Rhyssa*, *Ephialtes*, etc., in which the stalk of the egg is directed towards the oviduct, and is the first portion of the egg to pass into the ovipositor canal. The prolongation of one end of the egg into an elongate stalk is directly connected with the problem of its passage down the ovipositor." Previous workers have described pedunculate eggs among the Cynipoidea, Chalcidoidea, Proctotrypoidea and Ichneumonoidea and in several cases where the stalk remains partially exposed through the skin of the parasitised host, it has been suggested that the stalk aids the young larva in respiration.

In the Hymenoptera the stalk may be at either the anterior or posterior end of the egg but, although neither the emergence of the egg from the ovipositor of *Lyctus* nor the shape of the egg in the oviduct or ovipositor could be seen, it was easily proved that the strand was present on the anterior end of the egg by allowing beetles to oviposit in a sample which had had the pores sealed with paraffin wax except on one surface. When the sample was cut up, the eggs present all had the strand end pointing towards the unwaxed surface of the sample.

EGG PERIOD AND EMBRYONIC PHASE.

At this laboratory, Fisher (12) has already determined approximately the incubation period of the egg of *L. brunneus* without disturbing the egg in the wood. It was decided, however, to repeat this work with eggs removed from the wood in an attempt to establish the egg period within closer limits. Small samples of recently seasoned oak sapwood, $1 \times \frac{1}{2} \times \frac{1}{2}$ in., were exposed overnight to attack by a number of beetles. These samples were carefully shaved away with a razor and eggs were located with the help of a low-powdered binocular microscope. One or two eggs were removed from each sample and transferred to a glass slide. The time of laying was known to within about 12 hours and the development of the embryo could be followed by daily observation until the eclosion of the first stage larva. There was considerable variation in the incubation period for individual eggs. At 20° C. and 75 per cent. relative humidity (maintained by sulphuric acid in a desiccator) and also at 22·8° C. and 86 per cent. relative humidity, most eggs hatched in 8–8½ days, but in some cases the period extended up to 13 days. At 25° C. eggs also hatched in about 8 days, but at 30° C. only one egg out of 16 hatched, the time required being 7 days; of the remaining 15 eggs kept at 30° C. 7 had partly developed embryos within. 30° C. appears to be near the upper limit of temperature for development of eggs of *Lyctus brunneus*, but Fisher (12) obtained successful development of eggs at 32° C.

The changes which are visible within the egg (Fig. 9) during the period of incubation may be described briefly as follows:

First day. No visible change.

Second day. Slight increase in size of yolk globules, especially in anterior half of egg.

Third day. Yolk globules agglomerated into polygonal masses separated by distinct walls: posterior half of egg rather more transparent than anterior half.

Fourth day. Egg showing a more transparent portion near the middle; darker streak appearing in posterior half.

Fifth day. Very definite transparent area near middle of egg; embryo streak more distinct.

Sixth day. Median transparent portion larger.

Seventh day. Usually no change, though occasionally the larval outline becomes visible.

Eighth day. Larva hatched.

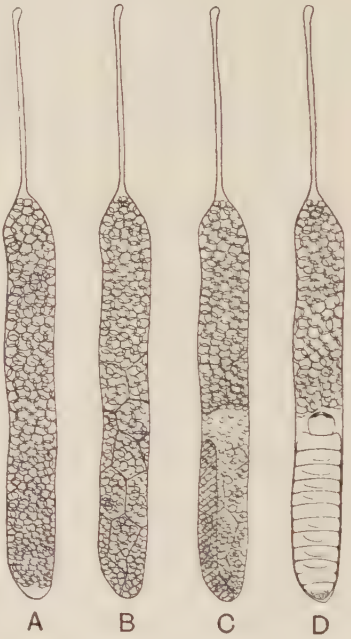

Fig. 9. *L. brunneus*. Embryonic development. A, 1st day; B, 3rd day; C, 5th day; D, 7th–8th day.

There appears to be a very sudden change during the last day, for on the penultimate day of development there is frequently no sign of the outline of the fully developed first stage larva. It is to be noted that about one-half of the yolk in the egg is not utilised during embryonic development and has been called by Altson (2) the "residual yolk mass." Abnormal eggs such as have already been described as freak eggs never hatched, nor, indeed, was any sign of embryonic development seen in them.

It is unsafe to outline the method of hatching as observed from eggs taken out of the wood vessels, since this process may be influenced by the leverage normally obtainable by the larva on the wall of the vessel. When eggs are exposed on a glass slide the larvae break the chorion posteriorly by means of two conical chitinised prominences and a series of smaller chitinised tubercles on the dorsal side of the ninth and tenth abdominal segments and then back out of the egg leaving the residual yolk mass untouched. According to Altson (2, 3) when eggs are not removed from the wood, the young larva eats its way out through the anterior end leaving the strand and anterior end of the chorion in the pore. Three to five days are taken to consume the residual yolk mass, unless the support of the walls of the vessel be removed, in which case the larva backs out of the egg as described above. In any case, the posterior end of the chorion is broken, possibly to allow access of air for respiration whilst the larva is feeding on the residual yolk. Kojima's observations help to confirm Altson's description.

VESSEL DIAMETER IN RELATION TO OVIPOSITION.

There are a number of factors influencing the liability of a timber to attack by *Lyctus* but among the most important is the question of the relation between the vessel diameter of a wood and its liability to attack. Fisher (12) and Clarke (9, 10) conclude that "pore size must be regarded as a limiting factor in the attacks of *Lyctus*," and show graphically and diagrammatically the relationship between range of vessel size of a number of timbers and their liability to *Lyctus* attack. A distribution curve of vessel diameters is given for each species.

According to this theory sycamore should not be attacked at all by *Lyctus*, whilst willow might be attacked very rarely. Cases of *Lyctus* attack in sycamore and willow have however recently come to the notice of the Forest Products Research Laboratory, and, in addition, experiments carried out at the Laboratory have shown that eggs can be laid and hatch in timbers which should be immune, or very nearly so. In most of the small-pored woods the young larvae died after boring a few millimetres, possibly because the wood did not contain suitable food material, *e.g.* starch (*vide* Wilson (23)), but the fact remains that eggs were laid in the small pores and hatched successfully. In Australian Crabapple (*Schizomeria ovata*) a small-pored wood with a range of vessel size similar to willow, the larvae completed their development and beetles emerged a year after infestation. Now it has already been stated that the egg is in all probability considerably increased in length and reduced in diameter during passage down the ovipositor and consequently, if the ovipositor is inserted into a vessel of smaller diameter than the normal diameter of the egg (see p. 504), the egg can expand only up to the diameter of that vessel and no more. This assumption has been substantiated by some measurements of eggs of *L. brunneus* laid in samples of willow. Since the eggs were tightly fixed in the vessels it was extremely difficult to remove them whole for measurement, but although the length of the egg body was obtained only in one instance ($1441\,\mu$)

it was in many instances evident that the eggs were considerably longer than the average for this species (1164μ). The diameter of the egg body was more easily measured; for 9 eggs this averaged 89μ, whereas the average for eggs laid in large-pored woods was 142μ. Corresponding to the increase in length of the egg body the strand is shorter on eggs laid in willow, the average strand length for four eggs being 142μ against the normal average of 418μ. The egg, then, is capable of having its normal dimensions altered in order to fit into a restricted space.

The original relationship between pore-size and egg diameter developed by Fisher and Clarke must be modified in order to allow for this adjustment of the diameter of the egg to the diameter of the vessel in which it is laid. The limiting factor must depend on the relation between vessel size and diameter of the ovipositor for, so long as the ovipositor can be inserted into a vessel, it is apparently possible for eggs to be laid therein. Conversely, if the vessels of a timber are so small that the ovipositor cannot be inserted into them, no eggs can be laid in that timber and it will be immune from powder-post beetle attack.

The ovipositor is more or less cylindrical and, measured at a point about half-way between the tip and base of the chitinised portion, the average diameter from 34 measurements was 78μ and the minimum recorded was 56μ. This means that, among the woods of which the range of vessel diameter was measured by Clarke, only Horse Chestnut (*Aesculus* sp.) should be completely immune from *Lyctus* attack because the pores are too small. Cherry (*Prunus*) and Apple (*Pyrus*) should be attacked but rarely, since only beetles with ovipositors below the average in diameter can lay eggs in the largest vessels of these timbers. These observations also account for many of the records of *Lyctus* attack in timbers which according to Fisher and Clarke's theory should have been immune, or nearly so, *e.g.* willow, Australian Crabapple, etc.

The measurements of the ovipositors for three species are given in the following table:

Table III.

Measurements of ovipositors of various species of Lyctus.

Species	Average diameter	Minimum diameter	No. of beetles measured
L. brunneus	78μ	56μ	34
L. linearis	83μ	73μ	10
L. planicollis	76μ	56μ	19

CONCLUSIONS.

The chief conclusion reached as a result of the work set forth in this paper is that the relationship between vessel diameter and liability to attack discovered by Fisher (12) and Clarke (9, 10) requires modification in the light of instances of *Lyctus* attack in small pored timbers which, according to their theory, should be immune. The theory must be restated in order to make it clear that the liability of a timber to *Lyctus* attack depends on the relationship between the vessel diameter of the timber and the ovipositor diameter of the beetle.

SUMMARY.

1. The life history and habits of the *Lyctus* powder-post beetles have been described and the sex ratio determined.

2. External sex characters are given for six species reared from timber at the Forest Products Research Laboratory, Princes Risborough.

3. The male and female reproductive organs of various species are briefly described.

4. An account is given of copulation and oviposition.

5. A detailed description is given of the egg of different species, before and after being laid.

6. The changes in form which probably occur as the egg passes from the calyx to the exterior are described, and evidence is given to support the statements made.

7. The period of incubation of the egg has been determined and observations are recorded on embryonic development and the eclosion of the larva.

8. Special attention is given to the modification of Fisher and Clarke's pore-size theory to fit the results of the present investigation.

ACKNOWLEDGMENTS.

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ON THE BIONOMICS AND STRUCTURE OF SOME DIPTEROUS LARVAE INFESTING CEREALS AND GRASSES

II. *OPOMYZA GERMINATIONIS* L.

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(With 5 Text-figures.)

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I. INTRODUCTION.

ALTHOUGH *Opomyza germinationis* L. is one of the commonest flies that may be swept from grasses and cereals, very little seems to be known of its habits and life history. Meigen (3) records the species from June to October in moist grasses and woods, Macquhart (2) also records it from woods. Zetterstedt (7) states that the fly is common on grasses and flowering plants, and in woods in Scandinavia; Walker (6) records it as being abundant in Britain and Schiner (4) records flies of the genus *Opomyza* "in endless number" from meadowland. A short description of the biology of the species is given by Czerny in Linder's *Die Fliegen der Palaearktischen Region* (1).

The methods employed for studying the species were the same as those for *Opomyza florum* Fabr. (5). Infested tillers of cereals and grasses were collected and planted in pots in the greenhouse and others were caged in the open. Records of times of emergence of the flies were taken from cages and by sweeping in the field. For observations on oviposition, flies were caged in hurricane lamp glasses with muslin tops, placed over seedling cereals and grasses.

II. Bionomics.

(*a*) *Field observations.*

In the summer, adults of *O. germinationis* may be swept in large numbers from grasses in meadows and pastures or along hedgerows and roadsides. In some seasons they fly from mid-June until mid-November, and in 1931 they were taken as early as June 10th and as late as November 11th.

First-instar larvae were found in grasses during October and early November, second-instar larvae from the end of October until the beginning of February, and third-instar larvae from the end of November until mid-June. Larvae were taken from *Poa trivialis*, *P. annua*, *Dactylis glomerata* and *Lolium perenne*. The injury to the grasses was very similar to that caused by the larva of *Oscinella frit* L., the central shoot being severed near the base. Larvae were also obtained from wheat and oats.

On April 4th, 1931, an examination was made of a field of wheat at Bottisham (Cambs.): in 1930 the field was under sainfoin, the crop was poor, one end of the field having such a thin crop that in July it was ploughed up and mustard sown; in the following autumn (1930) the whole of the field was ploughed and wheat sown. That part of the field in which mustard was not sown was attacked by larvae of *Leptohylemyia coarctata* Fal., the other part bore a good plant. On April 15th the majority of the wheat bulb fly larvae had pupated, but a number of third-instar larvae of *Opomyza germinationis* were found in the wheat, causing injury similar to that caused by the larvae of the former species. Later it will be shown that in all probability the adults had oviposited near the wheat after it had germinated in the autumn.

From another field of winter wheat at Bottisham, second- and third-instar larvae were taken on November 25th, 1931 and from wheat at the University Farm, Cambridge, second-instar larvae were taken on December 19th.

On April 13th, 1932 a census of the insect population of spring oats at the University Farm showed that in 1190 seedlings dug up from a hundred 10-inch lengths of rows, taken at random, there were present two third-instar larvae of *Oscinella frit* and four third-instar larvae of *Opomyza germinationis*. It is probable that the latter larvae had migrated from grasses into the oat seedlings, since no adults were present from the date of sowing of the oats (February 11th) until it was examined (April 13th).

(*b*) *Laboratory observations.*

In 1931 and 1932 adults were caged on various grasses and cereals. In preliminary observations in 1931 the only grasses and cereals used were wheat, *Poa trivialis*, *Lolium perenne* and *Dactylis glomerata*, but in 1932 a further series of pots was set up which included, in addition to the above, oats, *Lolium italicum*, *Cynosurus cristata* and *Festuca rubra*. Five pots of each of these plants were used, 20–30 seeds being sown in each pot. In addition, pots containing the following were also set up in duplicate:

Pot 1. Wheat, *Lolium perenne*, *Dactylis glomerata* and *Poa trivialis*.
Pot 2. Barley, *Lolium italicum*, *Festuca rubra* and *Poa trivialis*.
Pot 3. Oats, *Cynosurus cristata*, *Dactylis glomerata* and *Poa trivialis*.
Pot 4. Wheat, *Festuca rubra*, *Lolium perenne* and *Poa trivialis*.

Ten seedlings of each were planted in a pot and two male and two female flies were isolated over each pot about 7–14 days after the plants had come above ground.

In 1931 flies were caught and caged over the seedlings at intervals from the time when they first appeared in June, until September. The first date on which eggs were obtained was September 5th and subsequent observations in 1932 and 1933 showed that the egg-laying period of this species extends from the first week in September until early November. Both in 1931 and 1932 eggs were laid in various positions, some on the grasses and cereals at various heights on the stems and leaves, some on the soil and some on the lamp glasses; the majority however were laid on the stems of the plants near the ground.

The time taken for the eggs to hatch varies from 12 to 18 days. On hatching, the larvae crawl over the seedlings and feed indiscriminately on the plant tissues; after four or five hours they begin to bore down under the sheaths, generally from the top downwards until they reach the base of the central shoot where they settle down to feed. First-instar larvae were found in all the grasses and cereals sown. In the laboratory the larvae remained in the first instar for a period varying from 8 to 20 days, in the second instar, from 17 to 30 days and in the third instar, from 8 to 14 weeks. The pupal period occupied from 19 to 30 days. Flies hatched out from all the grasses and cereals sown, the greatest amount of injury occurring in *Poa trivialis*, every seedling being affected and the central shoot withered. This high infestation is accounted for by the fact that the larvae began to migrate from one seedling to another as early as the second instar. The cereals did not suffer so seriously as the grasses because they grew more strongly and consequently there was less migration of the larvae. Pupation took place in the seedlings near the ground.

From the above observations and from those made in the field, it appears that the larvae after hatching in early winter grow very slowly and pupate in May about a month before emergence.

III. Structure.

(*a*) *The third-instar larva.*

(i) *Morphology.*

The third-instar larva is about 6 mm. long and about 0·9 mm. in diameter. Compared with the larva of *Opomyza florum*, it is more elongated and more pointed at the anterior end. In colour it may vary from light green to white (the green coloration is probably produced by the chlorophyll in the plant tissue ingested). The segmentation is typical of Acalypterate larvae, there being a head, three thoracic and eight abdominal segments.

The head (Fig. 1) is small and rounded and may be retracted within the prothoracic segment. It is divided ventrally by a longitudinal groove and smaller grooves divide the ventral surface into small polygonal areas. Anteriorly it bears a pair of two-jointed *antennae* (*a.*) and on its ventral surface a pair of maxillary palpi (*m.p.*) consisting of five small papillae within a lightly chitinised ring, outside which are two smaller sensory papillae. There is a small heart-shaped *labium* (*l.*) on the ventral surface of the head immediately below the dentate sclerite; it bears near its posterior border on either side two minute papillae, the *labial palpi* (*l.p.*). Other sensory organs on the head are a second pair of small palpi (*m.p.I.*) behind the maxillary palps, a pair of papillae on the outer margins and a pair near the mid-ventral line.

The anterior margin of each segment bears a series of small denticles (*c.d.*) which are larger on the thoracic than on the abdominal segments and larger on the prothoracic than on the meso- and meta-thoracic segments. The prothoracic segment bears twelve incomplete rows dorsally, ten laterally and six ventrally; the mesothoracic segment seven

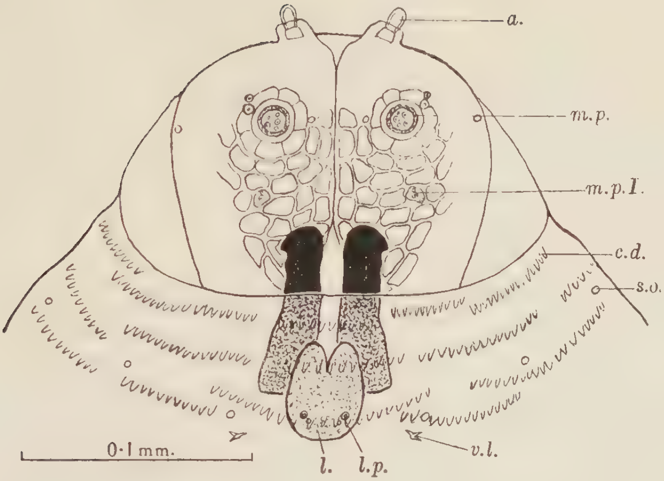
a.
m.p.
m.p.I.
c.d.
s.o.
0·1 mm.
l.
l.p.
v.l.

Fig. 1. Ventral view of head of third-instar larva of *O. germinationis*. *a.* antenna; *m.p.* maxillary palp; *m.p.I.* sensory palp; *c.d.* chitinous denticles; *s.o.* sensory organ; *l.* labium; *l.p.* labial palp; *v.l.* vestigial leg.

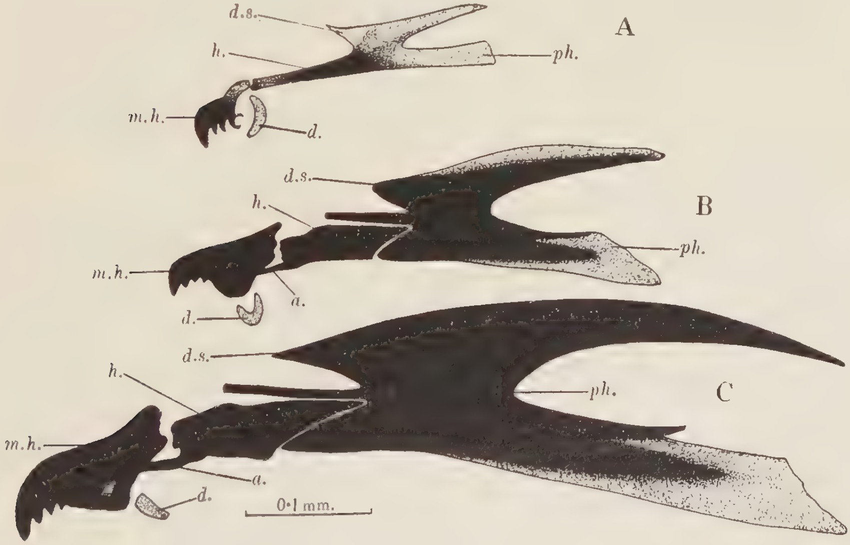
A
d.s.
h.
ph.
m.h.
d.
B
d.s.
h.
ph.
m.h.
a.
d.
C
d.s.
h.
ph.
m.h.
a.
d.
0·1 mm.

Fig. 2. Cephalo-pharyngeal skeletons of instars of larva of *O. germinationis*. A. First-instar larva. B. Second-instar larva. C. Third-instar larva—lateral view. *m.h.* mouth hook; *a.* accessory sclerite; *h.* hypostomal sclerite; *ph.* pharyngeal sclerite; *d.s.* median dorsal sclerite; *d.* dentate sclerite.

dorsally, four laterally and eight ventrally; and the meta-thoracic six dorsally, two laterally and six ventrally. The abdominal segments bear from one to four rows ventrally. Each of the thoracic segments bears a pair of vestigial legs (*v.l.*) near the mid-ventral line, each consisting of three hair-like processes which are swollen at their bases. There is also on each of these segments a transverse row of six minute sensory organs (*s.o.*) which appear as shallow pits.

(ii) *The cephalo-pharyngeal skeleton* (Fig. 2 C).

The *cephalo-pharyngeal skeleton*, compared with that of *Opomyza florum*, is slightly smaller and narrower. The component sclerites are—a pair of *mandibular sclerites* or *mouth-hooks* (*m.h.*), a pair of *accessory sclerites* (*a.*), a *hypostomal sclerite* (*h.*), a pair of *pharyngeal sclerites* (*ph.*), a *median dorsal sclerite* (*d.s.*) and a *median ventral* or *dentate sclerite* (*d.*).

Each mouth-hook has a large apical tooth with three smaller teeth behind, it is heavily sclerotised and articulates with the hypostomal sclerite by means of a sclerotised rod—the accessory sclerite, which passes along the inside of the mouth hooks and is attached to the cross piece of the hypostomal sclerite. Each pharyngeal sclerite is large and comparatively long and narrow, it has an anterior projection which extends over the posterior projection of the hypostomal sclerite and posteriorly is divided into dorsal and ventral projections. The median dorsal sclerite connects up the dorsal regions of the pharyngeal sclerites and anteriorly projects a short distance over the hypostomal sclerite.

(iii) *The respiratory system.*

The larva is amphipneustic and the whole of the respiratory system is very similar to that of *Opomyza florum*. The *anterior spiracles* (Fig. 3 A) open out near the posterior margin and on the dorso-lateral surface of the prothoracic segment. To each spiracle there are generally eight digitate processes, but the number may vary from seven to nine; each process opens out distally through a chitinised ring, the *peritreme* (*p.*). The *stigmatic trunks* (*s.t.*) are long and narrow and lead into the *main dorsal tracheal trunks* which extend along either side of the body near the dorsal surface. They open out at the posterior spiracles which are situated at the end of a short stigmatic papillae on the posterior end of the last abdominal segment.

Each *posterior spiracle* (Fig. 4 D) has three spiracular clefts (*s.c.*) each surrounded by a *peritreme* (*p.*); the *stigmatic trunk* (*s.t.*) however, does not give off a separate branch to each cleft. Four series of branched

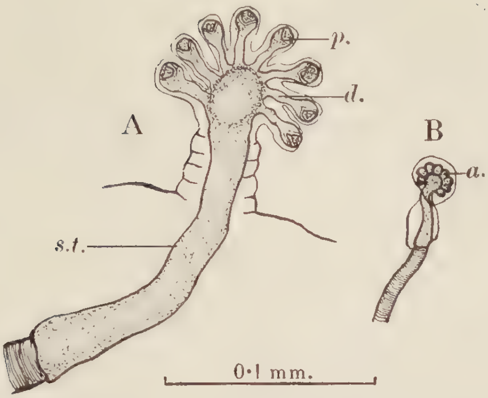
A
p.
d.
B
a.
s.t.
0·1 mm.

Fig. 3. A. Anterior spiracle of third-instar larva of *O. germinationis.* B. Anterior spiracle of second-instar larva. *a.*, *d.* digitate process; *s.t.* stigmatic trunk.

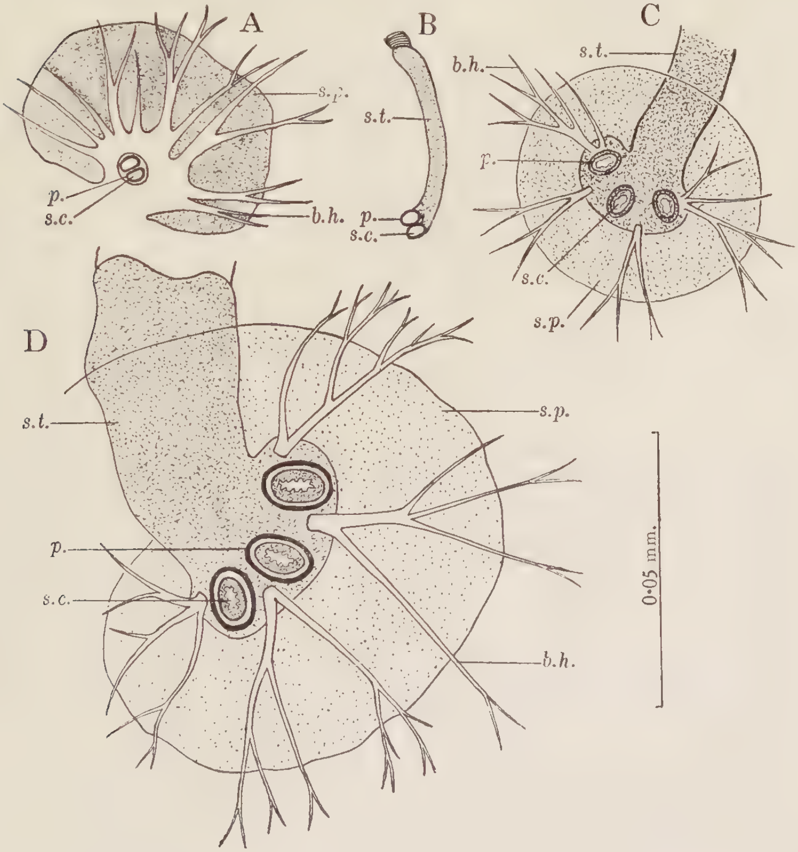
A
s.p.
p.
s.c.
b.h.
B
s.t.
p.
s.c.
C
s.t.
b.h.
p.
s.c.
s.p.
D
s.t.
s.p.
p.
s.c.
b.h.
0·05 mm.

Fig. 4. Posterior spiracles of larva of *O. germinationis.* A.B. First-instar larva. C. Second-instar larva. D. Third-instar larva. *s.c.* spiracular cleft; *p.* peritreme; *s.p.* stigmatic plate; *b.h.* branched hair; *s.t.* stigmatic trunk.

spiracular hairs (*b.h.*) arise between the spiracular clefts and extend over the stigmatic plate (*s.p.*).

Ten dorsal transverse commissures connect up the *main dorsal tracheal trunks*, the anterior and posterior commissures are thickened and have no tracheoles arising from them. There is a *secondary longitudinal trunk* from which tracheoles arise to supply the "brain" as described in the larva of *Opomyza florum* (5).

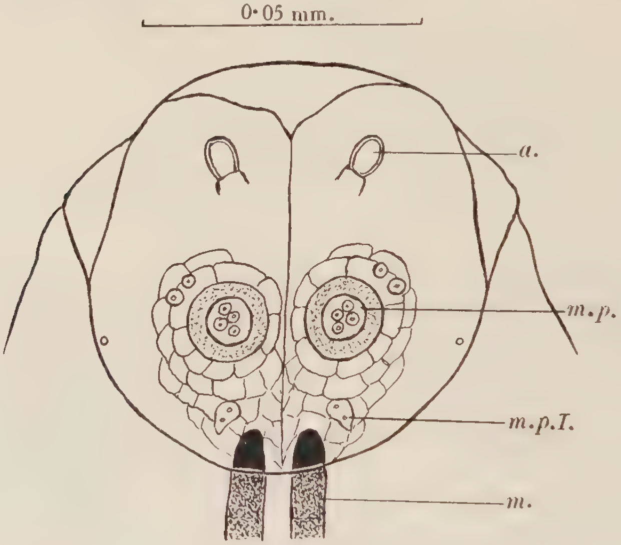

Fig. 5. Ventral view of head of second-instar larva of *O. germinationis*. *a.* antenna; *m.p.* maxillary palp; *m.p.I.* smaller palp; *m.* mandibular sclerite.

(*b*) *The second-instar larva.*

In the second instar, the larva varies from 1·8 to 2·9 mm. in length and is about 0·4 mm. wide. It is semitranslucent and its morphological characters resemble closely those of the third-instar larva.

The head (Fig. 5) bears a pair of two-jointed *antennae* (*a.*), a pair of *maxillary palpi* (*m.p.*), a pair of *smaller palpi* (*m.p.I.*) on the ventral surface and two pairs of sensory papillae which include a papilla on either lateral margin and a pair near the mid-ventral line. There is also a *labium* with *labial palpi* as in the third instar. The ventral surface near the maxillary palpi is divided into small areas, but these are not nearly so numerous as in the third-instar larva.

Each thoracic segment bears a pair of *vestigial legs* and the anterior margin of each segment, both thoracic and abdominal, bears broken lines of minute denticles as in the third-instar.

The *cephalo-pharyngeal skeleton* (Fig. 2 B) consists of a pair of *mouth-hooks* (*m.h.*) which are comparatively large and bear three teeth in addition to the large apical tooth, an H-shaped *hypostomal sclerite* (*h.*) and *median ventral* or *dentate sclerite* (*d.*), a pair of *pharyngeal sclerites* (*ph.*) and a *median dorsal sclerite* (*d.s.*). Its size compared with that of the first and third instars is shown in Fig. 2.

The larva is amphipneustic and the whole of the tracheal system resembles very closely that of the third instar; the main difference occurs in the structure of the *anterior spiracles* (Fig. 3 B). In the second instar the *digitate processes* (*d.*) are very short and the spiracle is very similar to that of the second-instar larva of *Opomyza florum*(5). There are two *main dorsal tracheal trunks*, a *secondary longitudinal trunk*, ten *dorsal transverse commissures* and a concentration of tracheoles at the "brain." Each *posterior spiracle* (Fig. 4 C) has three *spiracular clefts* (*s.c.*) but as in the third instar the *stigmatic trunk* (*s.t.*) is not divided into three branches. Four series of *branched spiracular hairs* (*b.h.*) arise between the stigmatic clefts and extend over the *stigmatic plate* (*s.p.*).

(*c*) *The first-instar larva.*

On hatching, the larva is about 1·1 mm. long but before the first ecdysis, it may attain a length of 2·2 mm.; its average width is about 0·24 mm. It is semitranslucent and tapers abruptly at the anterior end, its greatest diameter being in the region of the pro- and mesothoracic segments; from thence it tapers gradually to the last abdominal segment.

The number of *cephalic sensory organs* is the same as in the second and third instars; all are smaller but similar in structure. There is no reticulation of the ventral surface of the head.

In the *cephalo-pharyngeal skeleton* (Fig. 2 A) there is no differentiation into *hypostomal* and *pharyngeal* sclerites as in the second instar. There are two large *mouth-hooks* (*m.h.*) each articulating with a basal sclerite and having a large serrated apical tooth with three smaller teeth behind. Each basal sclerite consists of an anterior projection or *hypostomal region* (*h.*), corresponding to the *hypostomal sclerite* of the second instar, and a posterior or *pharyngeal region* (*ph.*). The pharyngeal portions are connected in the anterio-dorsal region by a lightly sclerotised strip which is in the position occupied by the *dorsal sclerite* of the later instars.

In this instar the larva is metapneustic. There are no *anterior prothoracic spiracles*. The *posterior spiracles* (Fig. 4 A) are small; each opens out through *two* spiracular clefts (*s.c.*) on small stigmatic papillae on the last abdominal segment. *Branched hairs* (*b.h.*) arise near the spiracular clefts and extend over the *stigmatic plate* (*s.p.*). From the posterior spiracles two main *dorsal tracheal trunks* extend forward and terminate in tracheoles in the region of the thoracic segments.

(*d*) *The egg.*

The egg is very similar to that of *Opomyza florum*, being subfusiform, flattened ventrally, and having a slightly curved dorsal surface. When laid it is about 0·56 mm. long and on hatching may attain a length of 1·05 mm. The white chorion is raised in longitudinal ridges most of which extend the whole length but some end abruptly, and its surface is covered with small bead-like papillae. There is a distinct plug-shaped micropylar area at the anterior end and at the posterior end the chorion is considerably thickened.

(*e*) *The puparium.*

The cylindrical puparium is similar to that of *Opomyza florum*. It varies in length from 3·85 to 4·3 mm. and is about 1·0 mm. in diameter. It is formed from the larval integument and thus retains some of the morphological characters of the larva. Larval spiracles are present at the anterior and posterior ends respectively, and the larval segmentation and denticles are retained. The anterior end in the region of the thoracic segments is not so abruptly flattened as that of *O. florum*.

Emergence of the adult is effected through V-shaped slits which open on either side of the thoracic segments, extending as far as the anterior margin of the first abdominal segment where a transverse slit occurs.

IV. SUMMARY.

In the laboratory, the larval food plants of *Opomyza germinationis* L. are, oats, wheat, barley, *Lolium perenne*, *L. italicum*, *Dactylis glomerata*, *Festuca rubra*, *Poa trivialis* and *Cynosurus cristata*. In the field, only a few larvae were found in the cereals, the chief host plants being the grasses. The injury is similar to that caused by the larva of *Oscinella frit* L.

There are three larval instars and pupation takes place inside the host plant near the ground. Adults emerge in mid-June and live until early November.

In the laboratory eggs were laid in September and October either on the plants generally near the ground level or on the soil near the seedlings.

A description is given of the three larval instars, the egg and the puparium.

The writer is indebted to Mr F. R. Petherbridge for help and advice and to Mr Collin for identification of the species.

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THE PINEAPPLE BUD MOTHS IN HAWAII

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Introduction.

The work described below was carried out at the Graduate School of Tropical Agriculture at the University of Hawaii, and the writer is much indebted for help and criticism to Prof. R. N. Chapman, Dean of the Graduate School and Director of the Experiment Station of the Association of Hawaiian Pineapple Canners.

The so-called bud moths occurring on pineapples in Hawaii constitute a group of insects the biology of which has been but little studied. They were considered to be of minor significance as pests, and attention has naturally been concentrated on insects known to be of primary importance. One species, *Pyroderces rileyi* Wlsm., was known to occur in greater or less abundance, but it was uncertain how many other species were concerned, what was the type and amount of damage done, or to what extent they were parasitised. Statements had been made that the larvae of *P. rileyi* were responsible for damage to living plant tissue and were the cause thereby of breakdown of the fruit through introduced rot organisms, a claim which, if correct, might justify the undertaking of control measures.

The present paper, in giving the results of an investigation into the bionomics of bud moths in pineapple fields, chiefly in the island of Oahu, represents an attempt to clear up some of the uncertain points in our knowledge of these insects.

Species concerned.

Swezey (23), in describing the bud moths of sugar cane, lists five species which were found by him on pineapples: *Ereunetis flavistriata* Wlsm., *E. minuscula* Wlsm., *Batrachedra rileyi* Wlsm. (=*Pyroderces rileyi*), and *Cryptoblabes aliena* Swezey. In the course of the present work only three of these species have been found, namely: *Ereunetis flavistriata*, *Pyroderces rileyi* and *Cryptoblabes aliena*. A close watch was kept among those specimens of *Ereunetis* which were bred in the laboratory for the occurrence of *E. minuscula*, but none were obtained.

Among the bud moths of pineapples occurring on Oahu, only two species need be considered as important, namely *Ereunetis flavistriata* and *Pyroderces rileyi*.

DESCRIPTION, LIFE HISTORY AND HABITS.

Swezey (23) gives an account of the life histories of *Ereunetis flavistriata* and *Pyroderces rileyi*, together with excellent descriptions and figures of the different stages of these insects, so that no more than a brief summary will be attempted here.

Ereunetis flavistriata Wlsm. (Fam. Tineidae).

"Antennae yellowish-white, with two small grey spots above before the apex. Palpi yellowish-white, brush-like beneath; the terminal joint very short. Head and thorax yellowish-white. Fore-wings yellowish-white, indistinctly streaked with broken yellow lines along the fold, along the cell, and below the costa beyond the middle; also sparsely speckled with black scales, especially beyond the middle; a short blackish streak at the upturned apex runs to the end of the apical cilia and is joined by a slender golden-brown streak along the base of the shining, white costal cilia; terminal cilia white cinereous, with a blackish spot in their middle below the apex. Exp. al. 14 mm. Hindwings shining, pale golden yellowish, becoming white at the apex; cilia pale yellowish-grey. Abdomen and legs yellowish-white." (Walsingham, *Fauna Hawaiiensis*, I, pt. v, p. 716, 1907.)

The moth may be immediately distinguished from *Pyroderces* by the sharply upturned tips of the fore-wings, its light yellowish grey colour and its larger size.

Attempts were made to induce these moths to mate and lay eggs in captivity, but without success. The females would lay unfertilised eggs, but no matings were obtained. The number of unfertilised eggs laid per female varied between 77 and 47 (from six females), with an average of 64·7 eggs.

Egg. Oval-oblong and very much flattened dorso-ventrally; reticulate, with parallel longitudinal carinae. Length about 0·75 mm., width about 0·25 mm. The eggs are laid singly, on young fruits and on the undersides of associated leaves, a favourite location being the basal leaves just below the fruit. The period of incubation is about two weeks.

Larva. The fully-grown larvae may reach a length of 15 mm.; they are greyish white in colour, though darker specimens are often met with, especially in decayed stumps. The larval period is rather long for an insect of this type; the exact duration is not known on pineapples, as none were reared through from egg to pupa successfully, but from observations on larvae brought in from the field which appeared to be in the first instar, it is probable that the time required is in the neighbourhood of six weeks or even longer. Swezey (23) mentions one which he reared in eight weeks.

Pupa. The pupa is enclosed in a dense silken cocoon in which frass and other debris are usually embedded. Prior to spinning, those larvae which have passed their life on fruits usually migrate on to the basal leaves before pupating, and the cocoon is very frequently spun in the hollow of a rolled up leaf. The pupa is characterised by a very pronounced laterally compressed protuberance in the front of the head, while posteriorly there are two small stout recurved processes on the dorsal surface of the cremaster, and two more, smaller and more conical, on its ventral surface. The pupal period is given by Swezey as "two or three weeks"; two were reared by the author in eleven and twelve days respectively.

In comparison with *Pyroderces* very little is known of the habits of this moth. Swezey (23) describes its occurrence in sugar-cane fields, where it is a general trash feeder, living for the most part beneath dead and dry leaves, though sometimes eating the surface of the rind of soft-growing tissue; the larvae are also said to eat the "eyes" or buds of the cane—whence the name "bud moth"—and in so doing may cause serious injury by rendering the cane unfit for seed, and by affording an opportunity for the admission of fungus spores. It is also stated that they feed on dead leaves of palms, bananas, *Pandanus* and pineapples. Strong (22) mentions "*Ereunetis* sp." being intercepted on coconuts from Hawaii.

Pyroderces rileyi Wlsm. (Fam. Elachistidae).

"Head chestnut-brown; palpi widely divergent, whitish, with an oblique pale brown mark on each side near the end of the second joint, and two or three brownish spots on the sides of the apical joint. Antennae with white and fuscous annulations; the basal joint elongate, chestnut-brown. Forewings chestnut-brown, slightly shaded with fuscous towards the costal margin; a whitish-ochreous streak at the base of the dorsal margin, followed by two or three other smaller ones along the dorsal margin (in some specimens these are obsolete); above the dorsal margin are two oblique whitish-ochreous streaks, the first before the middle, the second before the anal angle. A similar streak from the costal margin immediately before the apex is outwardly margined by a streak of black scales, the apex and apical margin being also black; there is also a faint fuscous streak running downwards through the cilia below the apex. On the cell are two elongate patches of black scales, one immediately before the middle of the wing, the other halfway between this and the base. Fringes grey, with a slight yellowish tinge. Hind-wings pale greyish. Hind tibiae greyish-white, outwardly fuscous; hind tarsi whitish, with a wide fuscous band followed by two fuscous spots on their outer sides. Expanse 11 mm." (Walsingham, *Trans. Amer. Ent. Soc.* x, 198, 1882.)

As with *Ereunetis*, all attempts to mate these moths under observation were unsuccessful, and it was found that unmated females would not, as in the case of the former, lay unfertilised eggs. One female, however, was obtained which had mated overnight before being isolated. This moth laid 22 eggs, of which 10 hatched. Another female which died without producing eggs was found on dissection to contain 23 ripe eggs. In addition to this, 32 eggs were obtained from a large number of females confined with males overnight in a glass vessel; 23 of these eggs hatched, after three days, but all attempts to rear the young larvae were unsuccessful.

Egg. The eggs are small, about 0·5 mm. in length, whitish in colour, oval and reticulate. They are laid singly, often near together, on young fruit, etc. The incubation period is three days.

Larvae. The larvae are bright pink in colour from the time of hatching until pupation. The head and cervical shield are brown, lightish at first, but darkening with age. They are smaller than the larvae of *Ereunetis*, being about 8 mm. in length when full grown.

Pupa. The pupa is surrounded by a silk cocoon, which is almost always constructed among the web and frass where the larva has fed. It is 4–5 mm. in length, the terminal segment bearing a number of stiff hooked hairs. The pupal period is from twelve to thirteen days.

Pyroderces rileyi Wlsm., also known as the Pink Corn Worm, is only in part a scavenger. It has been reported several times damaging corn, eating both cob and kernels in the field (Swezey (23)); in stored corn and sorghum (Chittenden (7)); in growing and stored corn in New South Wales, Australia (Gurney (11)). Back (1) mentions that it feeds on young kernels, cobs and husks. Harned (12) describes a serious outbreak on growing corn which occurred in Mississippi. Berger (4) records it on dried castor oil beans in Florida. The moth is also found in large numbers in cotton bolls, though usually in old and diseased ones, and is reported from many localities; in the United States (Chittenden (7), Busck (6)); in Peru (Da Costa Lima (9), Hinds (13)); in the West Indies (the Virgin Islands) (Wilson (27)).

Da Costa Lima (10) claims that *P. rileyi* is synonymous with *P. simplex* Wlsm., which has been reported on cotton bolls from Brazil (Da Costa Lima (8)) and several times from Africa, where Willcocks (26), Penzer (19) and Lamborn (17) record it on old or diseased bolls, while Morstatt (18) found it on sound bolls and stated that in German East Africa (now Tanganyika) it was at that date as serious a pest as the Pink Boll Worm.

Cryptoblabes aliena Swezey (Fam. Phycitidae).

"This moth with wings spread is 14–16 mm. long; grey, thickly irrorated with fuscous. Palpi grey, pale fuscous on outer side of second and third segments. Antennae pale grey. Head, thorax, and fore- and mid-femora with more or less crimson tinge. Abdomen pale grey brown. Fore-wings grey, thickly irrorated with fuscous, more or less suffusion of crimson at base, on basal half of costa, and on inner margin, also beyond the cell where it is sometimes confined to the veins; lines obsolete; sometimes a dark fuscous discal dot, and one or two dark fuscous discocellular dots; cilia grey-fuscous, darker at base. Hind wings pale and sub-hyaline, darker on veins, apex and hind margin; cilia whitish-grey." (Swezey (23).)

Two larvae only of this species were taken. They reach a length of about 12 mm. Swezey (23) mentions them as being "olivaceous, yellowish, reddish or fuscous," the colour apparently depending upon the type of food. The two specimens obtained on pineapples were dark fuscous, and were fully grown.

The pupa is formed in a sparse cocoon of coarse silk threads, which in both individuals observed was free from frass particles. The pupae are 6–7 mm. in length, light brown in colour, with a greenish tinge on the wing- and leg-cases. The cremaster bears two stout straight spines pointing backwards and hooked at the tip; these become firmly entangled in the silk of the cocoon and serve to anchor the pupa. Both specimens emerged after twelve days.

OCCURRENCE AND BIONOMICS ON THE ISLAND OF OAHU.

The first specimens of bud moths were obtained from an abandoned ratoon field (No. 16, Wahiawa), which was later knocked down. Both species (*Ereunetis* and *Pyroderces*), were present in considerable abundance, being particularly numerous on the young fruits. An idea as to the relative proportions of the species may be obtained from the numbers of moths which emerged from fruits brought in from the field and kept under observation in the laboratory. About two dozen fruits from this field yielded the following numbers of moths: *Pyroderces rileyi*, 157; *Ereunetis flavistriata*, 88; *Cryptoblabes aliena*, 2.

Later observations, however, gave noticeably different results, for *Pyroderces* was nowhere found again in such abundance.

The observations that follow are based on a survey of the island involving examination of fields in the following localities: (1) Wahiawa and neighbourhood, (2) Robinson, (3) Redhill, (4) Kahuku, (5) Kupihau.

Districts 1 and 2 are representative of the main pineapple growing area on Oahu; Nos. 3 and 5 are on more elevated land, the latter being a comparatively dry region. No. 4 is a more or less isolated district in the northern corner of the island.

(1) *Distribution.*

Ereunetis flavistriata. Distributed fairly evenly over the island of Oahu, its abundance in any one field or area being dependent upon the age or condition of the plants (see below).

Pyroderces rileyi. Generally distributed, but except in a few isolated places, not so abundant as the foregoing species.

(2) *Type of plant preferred.*

It has been stressed in the literature that these moths are essentially scavengers, living for the most part on dead plant tissue. This statement is at least partly borne out by the infestation observed on pineapples. The greatest numbers of larvae are always to be found in the withered stumps which are typical of old or abandoned ratoon fields. Larvae of all stages may be found in such stumps and at the bases of associated leaves, thus making it clear that the whole life cycle may be passed in such material, without the larvae having recourse to fruits at all.

On the other hand, larvae of all stages may be found occasionally at the bases of growing leaves of perfectly healthy plants where there appears to be almost no dead organic material, and it would seem that the growing tissue of the leaf itself must provide the source of nourishment. In such cases, however, repeated microscopic examinations of the leaf surfaces have failed to reveal any signs of attack. It is possible that such larvae have moved either upwards from the dead basal leaves or downwards from the fruit. It is perhaps significant that pupae are rarely if ever found at the base of healthy leaves.

Larvae of both species are also to be found in numbers on young pineapple fruits. The first eggs are apparently laid towards the close of the flowering stage, though oviposition may be continued for a considerable time, as evidenced by the occurrence of very young larvae on almost fully grown fruit. However, in the majority of cases, infestation is confined to the young fruit.

The presence of larvae on a fruit is clearly indicated by the abundant covering of web which they produce and under which they live. This web is always covered with frass and debris, sometimes to such an extent as to resemble the earthen coverings built by ants for the protection of their mealy-bug colonies (see under "Relations with Ants").

Illingworth (15) states that the larvae of *Batrachedra rileyi* (=*Pyroderces rileyi*) are "particularly troublesome on the fruits. They...frequently gnaw the leaves of the crown. Rot organisms enter through the wounds, causing the breakdown of the fruit." Examination of a very large number of young fruits in the field has failed to show evidence in support of this claim. Larvae of *Pyroderces* and of *Ereunetis* may be found frequently among the leaves of the crown, but in no single instance has the epidermis of a living leaf been seen to have been ruptured by them. The only apparent sign of feeding is the removal of the fine layer of dead cuticle which exists on the underside of the leaves. Furthermore, the very large percentage of healthy ripe fruits which, by the presence of web in the crown and elsewhere, show signs of previous infestation by bud moth larvae, furnishes further proof that these larvae are rarely, if ever, even an indirect cause of breakdown of the fruit.

(3) *Coincidence of bud moths with wilt disease.*

Wherever a bad outbreak of wilt occurs, especially in ratoon fields, bud moths are liable to be present in considerable numbers. There is, however, no evidence at all to indicate that they are responsible for the condition of the plants. In many severe outbreaks of wilt the fruits die and shrivel to form dry black fibrous "mummies," which may remain firmly attached to the plant for a long period. These provide ideal shelter and nourishment for the larvae, which may be present in large numbers, upwards of fifty having been taken from a single fruit. *Ereunetis* is almost invariably more abundant in such cases than *Pyroderces*, and is very frequently the only species represented.

(4) *Methods of distribution.*

A. *Flight.* The adults of both species are only weak flyers, especially in daylight. When walking among the plants moths may be disturbed and will be seen fluttering among and above the leaves, but no sustained flights are made. In the case of *Ereunetis* particularly, the adults tend to make short flights from plant to plant, settling instantly, and only moving when disturbed afresh.

No observations have been made in the field regarding their movements at night, but on entering the laboratory at night escaped moths have been noticed flying freely about the room, an occurrence which rarely takes place in daylight. It is possible, therefore, that more extensive flights may take place in the field after sunset.

B. *Wind.* Wind is probably the most effective agency in transferring moths from one area to another. The flight of the adults is so feeble that they would be incapable of resisting a strong wind, and if disturbed under such conditions they might easily be carried very considerable distances.

C. *Distribution in infested planting material.* Having in mind the quite frequent occurrence of bud-moth larvae in the "slips" growing from below infested fruits, the question arises whether larvae may be transferred in such material to be used for planting in a fresh district.

Frequent searches were therefore made in the collections of slips laid out to cure before being planted, and in newly planted fields. It was found that while occasional larvae occur in the curing material, they are very rarely discoverable in a field of young plants. Just what becomes of the larvae is still a matter for conjecture; it is possible that some may be killed by extremes of heat when the slips are exposed to continued sunshine, but the more probable explanation is that most are carried off by ants, *Pheidole megacephala* and *Solenopsis geminata*, which may be observed in numbers in and around the curing material. A few larvae probably leave the slips of their own accord and migrate to neighbouring plants. This migration of larvae has been observed in the laboratory, when similar infested material has been brought in from the field; the larvae on being disturbed become very active and may travel quite considerable distances in search of shelter.

(5) *Predacity.*

It is well known that certain Lepidopterous larvae are predacious in habit, and several authors mention Coccidae as being the objects of attack.

Beeson (2) refers to *Batrachedra sylvatica* Meyr. preying on the Chir Pine Scale (*Ripersia resinophila* Green) in India; Jarvis (16) found *Batrachedra* sp. attacking the San José Scale in Australia. Speare (21) states that *Pyroderces rileyi* occurs very freely among clustering grapefruit in Florida, where it was thought that the larvae were preying on mealy bugs. Experiments and field observations failed however to confirm this, and the author comes to the opinion that the larvae are only trash feeders. Berger (3) speaks of the same insect attacking *Icerya purchasi* in Florida, and Smith (20) records that it was bred from a consignment of Black Scale (*Saissetia oleae*) from Florida. There is apparently no mention in the literature of *Ereunetis* acting as a predator.

In view of the frequency with which both *Ereunetis* and *Pyroderces* are found associated with mealy bugs in pineapple fields, it was desirable to find out if they were acting in any degree as predators. No evidence was found to indicate such habits; in the field it is a common occurrence to find both species of larvae living among mealy-bug clusters, but careful search failed to reveal any signs of attack upon the latter, either in the form of partially devoured bodies or particles of wax in the frass of the larvae. In the laboratory, a number of larvae, taken from the vegetable refuse in which they were living, were provided with live mealy bugs of all ages, but made no attack upon them, continuing rather to feed upon the small amount of refuse present in the cage. It is clearly impossible to state definitely that these larvae never act as predators, but it seems certain that such a habit is not common, and would probably be of no consequence in contributing to the control of mealy bugs in the field.

(6) *Relationship with ants.*

The commonest ant in pineapple fields, *Pheidole megacephala* Fab., is well known for its practice of tending colonies of mealy bugs, and for its aggressive and omnivorous habits. It has been abundantly shown both in the field and in the laboratory that this ant will seize and kill any small lepidopterous larvae which may cross its path in the open, so that it is interesting to find bud moth larvae occurring freely with ants and mealy bugs on young pineapples. Such larvae are apparently not molested at all, in fact many instances have been noted where *Pheidole* has supplemented the silk web of the larvae with earth, to form a communal covering sheltering ants, mealy-bugs and bud-moth larvae. Under normal conditions in the field, therefore, *Pheidole* cannot be considered to be a controlling factor, but when the larvae are disturbed and wander from their normal locations, as for instance when "slips" are laid out for curing, there is evidence to show that this ant may perform quite an important function as a predator.

(7) *Natural enemies.*

A. *Parasites.* Both *Ereunetis* and *Pyroderces* found in pineapple fields are very largely free from parasites. Only two parasites have been bred from several hundred larvae, including both species, which have been kept under observation in the laboratory. These were both females of *Idecthis* sp., an Ichneumonid closely allied to *Nemeritis* (*Idecthis*) *canescens*, and previously bred from other species of Tineinae. Swezey first records the insect in Oahu as reared from *Stagmatophora* in 1928, since when it has increased considerably in numbers. Swezey (23) mentions two Hymenopterous parasites bred from larvae or cocoons of bud moths: *Melittobiopsis ereunetiphila* Timberlake (referred to as *Melittobia hawaiiensis* Perk.) bred from a number of cocoons of *Ereunetis flavistriata* collected in a cane field, and *Microdus hawaiicola* Ashm. bred on a few occasions from *Pyroderces rileyi* and once from *Ereunetis flavistriata*. Bridwell(5) records the Bethylid *Perisierola emigrata* Rohw. as attacking larvae of *Pyroderces rileyi* in captivity; Swezey(24) reared *Sierola molokaiensis*, another Bethylid, in quite large numbers from cocoons of *Ereunetis flavistriata*, and records *Cremastus hymeneae* Viereck parasitising both *Pyroderces rileyi* and *Ereunetis flavistriata* in Hawaii(25).

B. *Predators.* Bud-moth larvae are undoubtedly preyed on to a certain extent by various wasps, which may be seen in any pineapple field flying around the plants in search of prey, the most numerous being *Odynerus* sp. Illingworth(14) lists *Polistes aurifer* Sauss., *P. hebraus* Fab. and *Pachodynerus simplicornis* Sauss. as preying on larvae of *Pyroderces rileyi* in the Kona district of Hawaii.

Even though such predators may kill many bud-moth larvae, yet they probably reach only a small minority, for these larvae by their secretive habits and their practice of constructing dense silk webs are very well protected from attack.

(8) *Control measures.*

At the present time no special measures are taken for the control of bud moths. The general spray programme directed against mealy bugs, consisting of a proprietary oil spray applied at regular intervals, is, however, undoubtedly effective in reducing the numbers of bud-moth larvae on the fruits.

No spray is used in old ratoon fields, where the larvae are most abundant, but the practice of knocking down and discing such fields results in the almost complete annihilation of all stages, except possibly the adults. This has been shown by repeated examination of recently knocked down fields.

Such being the case, and in view of the very small amount of actual damage done by these insects, any further measures directed specifically against bud moths would probably be a waste of effort and expense.

(9) *Summary.*

1. The two most important species of bud moth in Oahu are *Pyroderces rileyi* Wlsm. and *Ereunetis flavistriata* Wlsm.

2. Both species are evenly distributed over the island, the latter being the more common.

3. They are most numerous in old ratoon fields, where they feed on dead plant tissue. They are also often found on young fruits, where, contrary to certain claims, they appear to do very little damage.

4. Methods of distribution are discussed; wind is probably the most important factor.

5. Although often found with mealy bugs, neither species appears to be predacious.

6. Except when disturbed, the larvae are not molested by ants, but when unfavourable circumstances cause them to wander, large numbers may be killed in this way.

7. Parasitism is very low. Only two parasites have been reared from several hundred larvae. Predators, principally Sphecoid wasps, carry off many larvae, but being unable to reach the majority are relatively ineffective as a means of control.

8. Apart from the regular spraying for control of mealy bugs and the knocking down of old ratoon fields, no specific control measures are practised against bud moths, and any further effort would appear to be unwarranted.

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AN AUTOMATIC HUMIDITY CONTROL

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(With 3 Text-figures.)

It is well known that humidity plays an important part in affecting insects' development, and many devices have been used for the control of humidity in small spaces.

Part of the work of these laboratories consists in breeding, on a large scale, various stored product insects to be used in biological and chemical experiments.

It was found that when breeding *Plodia interpunctella* Hb. (the Indian Meal Moth), *Ephestia cautella* Wlk. (the Fig Moth) and *E. elutella* Hb. (the Cacao Moth) it was desirable to maintain the atmosphere of the insectary at a fairly uniform humidity of about 75 per cent.

The humidifier here described was designed for this purpose. In principle it is somewhat similar to one in use at the Citrus Scale Laboratory at Whittier, California, but differs from it in many details, and in being automatic in operation.

The apparatus, Fig. 1, consists of a powerful electric fan (*A*), which also drives, by pulley and belt, a small centrifugal water pump (*B*). A jet of water from this pump rotates a large cylinder of perforated zinc (*C*), 18 in. long by 10 in. in diameter, which dips into a deep tray of water (*D*). Suitable baffles are fitted to this cylinder, and air is blown over the wet metal by the fan; the arrangement of these baffles ensures thorough saturation of the air.

One or two constructional details may be of interest. The side thrust of the belt drive to the centrifugal pump is taken up by a cycle hub, which is coupled by a piece of stout pressure tubing to the pump spindle. The jet of water from the pump rotates the cylinder by impinging upon sewing thimbles fixed to the cylinder and acting as the buckets on an overshot water wheel. Twenty thimbles were spot soldered to a strip of brass-wire gauze which was clipped round the cylinder. The cylinder itself is mounted in adjustable pin bearings.

Control. The control, Fig. 2, is automatic, and works on the principle of a recording hair hygrometer. Human hairs are attached at one end to a spindle (*a*), mounted in clamps for adjustment, and at the other are wound several times round and fixed to a second horizontal spindle (*b*), mounted in delicate pin bearings and fitted with an arm (*c*) and adjustable counterbalance (*d*). On the end of the arm are mounted two electrical contacts, facing up and down. Movement of the arm in a vertical plane is occasioned by the expansion or contraction of the hairs, and causes these contacts to strike either the upper or the lower of two

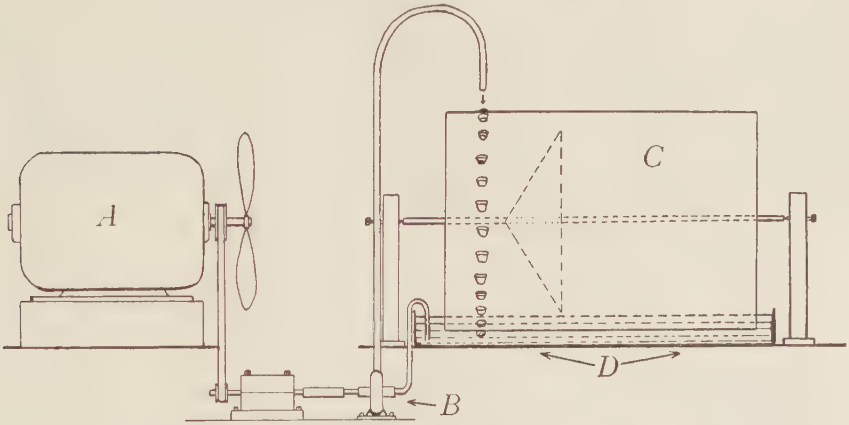

Fig. 1. Humidifier, showing fan, pump and revolving drum.

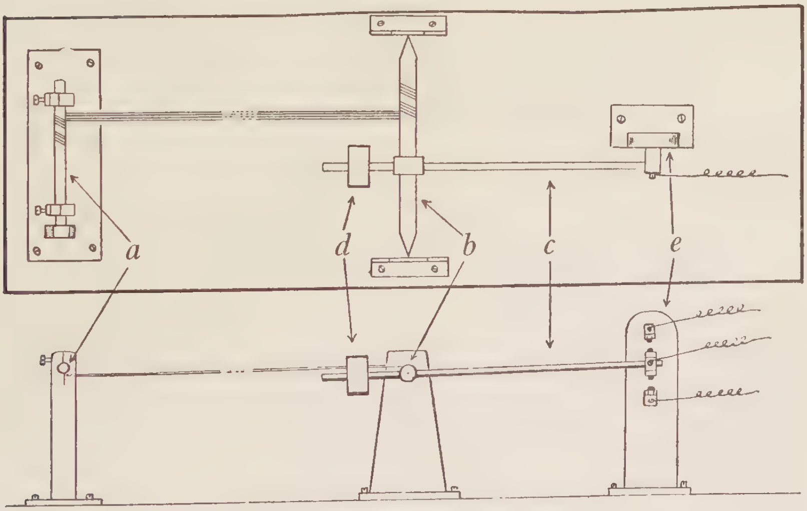

Fig. 2. Control mechanism, showing plan (above) and elevation (below).

fixed contacts arranged on a bracket (*e*), thereby completing an electrical circuit which either starts or stops the fan motor of the humidifier.

The control mechanism is erected in a different part of the insectary from the humidifier, and is in the draught of a small desk fan, which runs continuously.

Electrical circuit (Fig. 3). The contacts on the control mechanism make or break a current of five volts obtained from the mains through a small bell transformer. This current operates two electro-magnets taken from an electric bell, which move, by levers, a mercury switch controlling the main current of the fan motor.

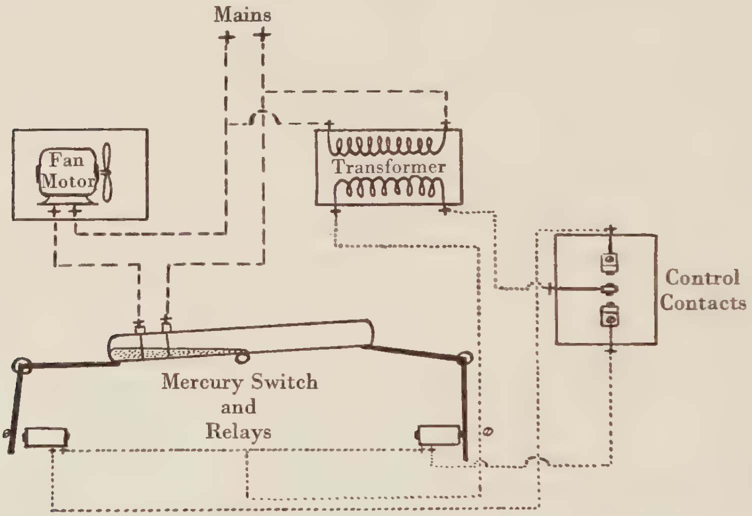

Fig. 3. Wiring diagram. – – – – =220 volts. .... =5 volts.

As the apparatus was originally constructed, there was only one contact on the control arm, which operated one relay, tilting the mercury switch to the "on" position, from which it was returned by gravity. It was found, however, that any vibration of the control arm caused the fan motor to switch on and off with great rapidity, which was harmful to the motor, so the second contact was installed with its own electro-magnet, and the mercury switch so pivoted as to swing well over when activated by either magnet, and to remain there until thrust back by the other. This obviated all tendency to chatter, and the motor, when once started, is able to run steadily until positively switched off.

Operation. The humidifier is required to condition the air in an insectary measuring about 600 cubic feet; there are three outside walls which are only one brick thick, and there is no rough cast or other protective covering.

With the temperature kept constant, the relative humidity varied ±2 per cent. (73–77 per cent.). With the temperature fluctuating, as it normally does in summer when the building is not artificially heated, the humidity will vary considerably (73–80 per cent. by hygrograph chart). This high variation is due to the fact that when warm air, conditioned to 75 per cent. relative humidity, is cooled down, the humidity inevitably rises.

Accurate control can therefore be obtained with an instrument of this type only when the temperature is kept constant.

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REVIEWS

Termites and Termite Control. Charles A. Kofoid, Editor in Chief. Pp. xxv + 734, 182 figs. Berkeley University of California Press. 1934.

The United States supports an unusually large termite fauna for a region lying mainly outside the tropics. While only two species of these insects occur in Europe, a total of fifty-five species is already known from America, north of Mexico. Further study will doubtlessly reveal forms as yet undiscovered, particularly on the southern border and coastal states, where an influx of tropical species from Mexico has evidently occurred. The American species include representatives of each of the termite families (excepting the Mastotermitidae), the primitive group of the Calotermitidae having an equal number of species to the Termitidae, which are the dominant termites of most tropical countries. Since the monograph of Hagen, completed in 1860, these insects aroused little interest among American entomologists for many years. With the publication of independent observations by Snyder in 1915 a fresh impetus was given to their study and, in 1920, Banks and Snyder contributed their important revision of the Nearctic species. During the last few years a good deal of attention has been given to the termites of the Pacific coast area, chiefly because the damage wrought by these insects has become increasingly evident in this region. In 1928 a special "Termite Investigations Committee" came into being and, since it has been generously supported by grants from various commercial organisations, it has been able to formulate and carry out a very considerable amount of inquiry. A report of this Committee in the form of a book entitled "Termites and Termite Control" was issued in January of the present year. Under the general editorship of Prof. C. A. Kofoid it embodies the results of investigations carried out by over thirty collaborators—each contributor being separately, or jointly, responsible for one or more of its chapters. This volume, which extends to over 730 pages, is admirably written and illustrated and is broadly divisible into two sections. The first part (pp. 1–335) comprises a series of chapters discussing the general biology of termites together with an account of the species found in North America, the Canal Zone, West Indies and the Hawaiian and Philippine Islands. The reader will find in these pages a very clear and up-to-date account of termite biology and structure with numerous original illustrations. While it does not pretend to probe deeply into any of the fundamental problems of termite life, it brings together a good deal of ecological data not previously published, and numerous facts bearing on the behaviour of different species. The second part of the book is devoted to recommendations for the prevention and control of termite damage by diverse means: improved methods of building construction are dealt with at length together with insecticidal measures. The economic entomologist will welcome the volume since it provides the most exhaustive account of methods for the contravention of termite damage so far available. The large amount of new information by way of tests, and recommendations based on those tests, make the book one of special importance to all and sundry who are concerned with termite control, not only in America, but in other parts of the world.

A. D. IMMS.

Virus Diseases of Plants. By John Grainger Pp. viii + 104, with Plates VI. Oxford University Press. 1934. 6*s*.

Although so new a development, virus diseases of plants have assumed an importance which requires their inclusion within any teaching course designed to introduce students to the subject of disease in plants. In most recent text books of plant pathology there are descriptions of a greater or lesser number of actual virus diseases of specific hosts but in no text book is the subject treated from a methodical point of view as a distinct branch of the science of plant disease. In the course of his teaching work, Dr Grainger found "the need for a small book upon the subject of Plant Virus Diseases," and in so far as it goes his little book meets this need and will serve as an introduction to the practical study of virus diseases. It is however a little difficult to see exactly for whom the book has been written. It is not very suitable for an undergraduate student, or for use in a general class course in plant pathology, whilst, although an honours student or a post-graduate student commencing research on virus diseases may find the book useful, he will soon put it on one side and go to original papers.

Following a very short historical introduction, Chapter II is devoted to a consideration of the relation of a virus to its host plant. Chapter III deals with the properties of virus extract, Chapter IV with the relation of insects to virus diseases, Chapter V with economic effects and control measures, Chapter VI with the classification and description of virus diseases and Chapter VII with general experimental work. The book concludes with a bibliography of 445 references and an index.

WILLIAM B. BRIERLEY.

Plants and Human Economics. By RONALD GOOD. Pp. xii+202, with 8 maps. Cambridge University Press. 1933. 5*s.*

In his preface the author very rightly emphasises the remarkable fact that "the aspect of botany most intimately concerning human affairs and activities, economic botany, is the aspect least taught." He has therefore endeavoured to "combine in small compass and, it is hoped, in reasonable and readable fashion, not only the botanical facts but also, and frequently more important, the historical and economic facts required to give to those who begin the scientific study of botany an adequate humanistic background of reality to their subject." He has in fact tried to lay before students "the evidence that the science of plants is something more than a mere mental discipline." The "book is intended primarily for those in the senior classes of schools and the junior classes of universities," but the author has tried "to make it also of potential value to those who do not happen to be students of biology" by making "the later chapters as complete as their size permits so that they can be used to some extent for reference."

The first five chapters deal generally with plants in relation to human civilisation, the nature and sources of food, the life of the green plant, factors limiting agricultural production and science and agriculture. This portion is well and interestingly written and serves as an excellent introduction to the more technical aspects of economic botany.

Most of the remainder of the book deals specifically with economic plants and the commercial products derived from them, and the scope can best be realised from the chapter headings which run as follows: VI, Cereals and pulses; VII, Vegetables; salad plants; fruits; VIII, Beverages; sugar and starch oils and fats; spices; IX, Timber, coal and petroleum; X, Rubber; resins, balsams and gums; tans and dyes; fibres; XI, Alcohol; drugs; fodders; miscellaneous; XII, The useful products of the lower plants; concluding notes on vegetable products.

These chapters contain an astonishing amount of interesting and useful information but, owing to the exigencies of space, they tend to read rather like an annotated catalogue and at times are reminiscent of the Pharmacopeia. It is a great pity that the more expansive and interesting style of the first few chapters could not have been maintained. Incidentally the Conifer nomenclature is hardly adequate and in dealing with the origin of cultivated plants the recent Russian work might have been given more attention.

The last chapter, XIII, is a sketch of the economic botany of Great Britain in relation to its industrial and commercial development and quite effectively rounds off the book.

There is an interesting, but ineffective, "Selected Reference List," from which publication dates are omitted and an Appendix which is a combined list and index of the species and genera of seed plants mentioned in the book, arranged in systematic order, together with the common names applied to them or to their products. The book closes with a very inadequate index and is illustrated with eight distribution maps.

The book is an excellent and almost successful attempt to present a point of view which very badly needs presenting in our teaching institutions. As a little book of reference it would have been infinitely more useful had there been a more complete index and two other appendices, one an alphabetical list of plants with their products and the other an alphabetical list of products with their plants. Still it is a work that many botanists should find useful and one that will provide excellent reading and new viewpoints for students. Although its price is only five shillings the book is beautifully produced.

W. B. BRIERLEY.

PAUL SORAUER. *Handbuch der Pflanzenkrankheiten.* Herausgegeben von Dr O. APPEL. 6th edition. Vol. I. *Die nichtparasitären und Virus-Krankheiten.* Part I. (Non-parasitic diseases.) Pp. x+592. Berlin: Paul Parey. 1933. 46.00 gold marks.

The development of the science of plant pathology can well be gauged by comparing the several editions of Sorauer's great *Handbuch.* First published just 60 years ago as a single small volume it was immediately recognised as a work of outstanding quality. The second edition appeared in two volumes twelve years later and the third edition, comprising a monumental tome on the non-parasitic diseases, a much smaller volume on the bacterial diseases and a large new third volume on animal pests, was completed in 1913. The volumes of the fourth and fifth editions got a little out of step but were completed in 1931 when the work extended to five massive tomes. It was in the third edition that the work assumed its characteristic form. Vol. I of this edition, issued in 1909, was devoted to non-parasitic diseases and ran to some 890 pages entirely from the veteran albeit difficult pen of the original author. Sorauer died in 1916 and Vol. I of the fourth edition was largely rewritten by Graebner and increased in size by some 70 pages although the actual arrangement followed closely that of Sorauer's earlier work. Vol. I of the fifth edition, also written by Graebner, differed but little from its predecessor but was increased by some 21 pages. Thus *Die nichtparasitären Krankheiten* of 1924 is substantially the same as the volume issued in 1909.

The *Handbuch* has now passed under the sole editorship of our distinguished Honorary Member Dr Appel, and Part I of the sixth edition which has just appeared shows a notable change in structure and format. Vol. I of the fifth edition still retained the old title, *Die nichtparasitären Krankheiten,* but in the new edition this has been changed to *Die nichtparasitären und Virus-Krankheiten.* Vol. I is to appear in two parts of which the first, now published, deals only with certain aspects of non-parasitic disease. In Vol. I of the fifth edition the virus diseases received short shrift and were run along with the Gummoses into a short section at the end, under the heading "Enzymatische Krankheiten." The increased recognition of their special nature and importance, which is largely a post-war development, is shown by the devotion to them of a special Section in Part II of Vol. I yet to appear.

Further, in the fifth edition Vol. I remained the work of one contributor but, for the new edition, Dr Appel has recruited various well-known German plant pathologists to deal with the several portions of the subject. In producing the third edition of the *Handbuch* Sorauer recognised that one man could no longer cover the whole subject of "Pflanzenkrankheiten" and sought the aid of Appel and Reh., and the process has now been continued even to the separate volumes. This course is inevitable but the gain in comprehensiveness and accuracy is often offset by a loss of coherence and unity. In the present case however Dr Appel's wise choice of his coadjutors and his careful editorship have produced a volume which reads almost as the work of one author.

The book is divided into two parts, a general part of two Sections and a special part of three Sections. Section I (80 pages) written by Braun of Stade is a fascinating account of the history of plant pathology from 4000 B.C. to about A.D. 1880. It is by far the best treatment the subject has yet received although one cannot but regret that Senn's later published Essay was not available to the author.

Section II (118 pages) by Morstatt of Berlin-Dahlem is a very good introduction to the study of the general phenomena and viewpoints of phytopathology. The first few pages really follow from the previous section and set out the more recent developments of the subject. An all too brief consideration of the nature of disease then leads to the classification and description of symptoms followed by a discussion of parasitism and pathogenicity. The second chapter of this section deals with plant ecology in its disease relationships, passing to a consideration of plant and animal parasites in relation to their environments. This latter subject has not received adequate notice from plant pathologists although its importance is widely recognised in economic entomology, and the treatment it is accorded here will usefully direct attention to it.

The remaining two-thirds of the volume constitutes the special part. The first section (120 pages), contributed by Merkenschlager of Berlin-Dahlem, is a detailed consideration of plant nutrition in relation to plant disease and is divided into a longer chapter dealing with general problems and a shorter one dealing with special problems. A most valuable feature of the former chapter is a detailed consideration of mineral deficiencies and their effect on the plant whilst the special diseases dealt with in the latter chapter are heart and dry rot of beets, grey fleck of oats, white-head of oats and grasses and moor disease.

The second section (157 pages), written by Hiltner of Munich, contains a full discussion of climatic factors as causes of non-parasitic diseases; light and radiation, temperature, water movement and wind being considered in turn. These factors are treated both in their direct and indirect relationships and the whole section is an exceptionally good and well-balanced résumé of our present knowledge.

The final section (117 pages) is contributed by Wartenberg of Berlin-Dahlem and deals with extremes of temperature as the cause of disease and death in plants, both in their direct effects and indirectly as laying the plant open to parasitic invasion.

The volume opens with a good synoptic "Contents," which is fortunate seeing that it closes without an index. No doubt the latter will appear at the end of the second part of Vol. I, but a scientific treatise of 592 pages published as an independent volume and packed with matter deserves an index of its own. There are 123 text illustrations, many being the original Sorauer figures. An increase in their number would have enlarged the book but the added value to the reader would have been an overwhelming compensation. References are given in footnotes which make it difficult to track individual papers, are largely German and include publications until about the end of 1931.

The non-parasitic diseases of plants is a major portion of the subject which has been considerably neglected by British plant pathologists. It is, of course, normal plant physiology seen in a special perspective and this has been, to a certain extent, obscured for us by reason of our mycological obsession, enshrined, for example, in our retention of the misnomer "mycology" for "plant pathology." The increased study of virus diseases and nutritional troubles in which we are compelled to get away from the fungi is, however, gradually bringing us to a more adequate viewpoint. German plant pathologists have always been more physiologically minded than have we in this country and this has been reflected in Sorauer's *Handbuch*. The present volume is really a treatise on special plant physiology, the keynote being the conditioning of the normal physiological processes of the plant, and the resulting normal morphological structure, by environmental factors and the plant's reactions to abnormally great variations in the impact, the rhythm and balance of these environmental factors.

This new edition fully maintains the great tradition of the old "Sorauer" and the Editor and contributors are to be congratulated on their achievement. The third edition of *Die nichtparasitären Krankheiten* was translated into English by Dorrance, but this is now considerably out of date and, as we have nothing in English approaching the present treatment of the subject, it is to be hoped that arrangements may be made for its translation. The volume, like so many other German books, is excessively priced.

W. B. BRIERLEY.

The Toxicology of Plants in South Africa. By D. G. STEYN. Pp. xii + 631. South Africa: Central News Agency, Ltd., 1934. (English copies from Gordon and Gotch, Ltd., Gordon House, 75–79 Farringdon Street, London, E.C. 4.) Price £2. 7*s.* 6*d.*

Owing largely to the activities of Sir Arnold Theiler, South Africa has long occupied a leading position in veterinary science. This is just as well since the country abounds in poisonous plants and more stock is lost annually from plant poisoning than from any other disease. In 1926–7 the loss from *Tribulus terrestris* L. alone in the north-western Cape Province was between 600,000 and 700,000 small stock, and in the south-eastern portion of South-West Africa and in Griqualand West, during the years 1929–30 *Geigeria passerinoides* took toll of over 1,000,000 sheep. It is to be expected therefore that considerable attention would be directed to the study of poisonous plants and, in addition to an extensive periodical literature, the results have appeared in the masterly volume on *The Medicinal and Poisonous Plants of Southern Africa* by Watt and Breyer-Brandwijk published two years ago (see Review in the *Annals*, xx, 3, August 1933) and in the present volume. The world over, knowledge of plant poisoning is in a very unsatisfactory condition, but no country has done more to remove this reproach than South Africa.

Dr Steyn divides his book into two portions. A general part of some 73 pages opens with a brief historical introduction and passes to discussions of the classification of poisons, their absorption, fate in the body, mode of action, toxic principles and physiological significance, and detection. These are followed by brief notes on the diagnosis and prognosis of plant poisoning, general principles of treatment, an interesting consideration of plant and animal factors concerned in the determination of the toxicity of plants, and the conditions giving rise to accidental ingestion of poisonous plants. There follow two elementary chapters dealing with eradication of poisonous plants and prevention of plant poisoning, and a note on the legal aspect of plant poisoning. The next few pages are perhaps more interesting and deal with the experimental investigation of plant poisoning. The general part closes with notes on losses from plant poisoning, the action of poisons on foetuses and the indirect poisoning of human beings by carcases of poisoned animals, by milk and by honey. All the foregoing is very scrappy and unsatisfactory but that is because the state of our knowledge is itself very scrappy and unsatisfactory. The position concerning stock poisons and especially plant poisons can perhaps best be gauged by comparing the state of knowledge reflected in this volume with that concerning human poisons as reflected in Cushny's *Pharmacology and Therapeutics*. The toxicology of plants to stock is still in its infancy and, if the present book brings this home to scientific workers and stimulates them to the further experimental research which is so greatly needed, the author will probably feel more than justified in his labours.

The remaining 500 pages of the volume are devoted to a consideration of the special toxicology of South African plants. The first section (66 pages) deals with poisonous foodstuffs, including those plants which, when taken in large quantities over prolonged periods, cause poisoning by virtue of toxic substances naturally contained in them and excluding those foodstuffs which are rendered poisonous by the action of microorganisms, chemical decomposition or contamination with poisonous plants. The natural orders are taken in turn and each species is considered exhaustively according to the following schedule: taxonomy; use as cattle food etc.; active principle; toxicity; symptoms of poisoning; post-mortem appearances and histology; treatment, prevention; discussion. This section closes with an admirable account of prussic acid poisoning in animals and man due to the ingestion of cyanogenetic Gramineae.

The second section (15 pages) is a valuable account of the interesting and obscure phenomenon of photosensitisation which results from the animal ingestion of certain plants. This state is sometimes produced by the eating of the common buckwheat by various mammals and birds but is most disastrously found in semi-arid regions where sheep feed on the Devil's Thorn (*Tribulus terrestris*). It is a field of study in need of much greater exploration.

Section three, two and a half pages in length, contains a description of the skin eruptions of cattle fed on potato tubers, leaves or residues. Many other plant residues such as grape skins, beet refuse, malt, molasses, rice bran and sesame meal may also give rise to eczemas but little is known of their etiology.

In the fourth section (34 pages) the fungi are considered in relation to health in man and animals. There is a valuable introductory chapter summarising literature of the last 30 years, which leads to a discussion of the toxic constituents of fungi and fungus infected foodstuffs and closes with a tantalisingly brief note on natural and acquired immunity to poisoning with foodstuffs infected by *Diplodia zeae*, *Gibberella Saubenettii* and *Aspergillus fumigatus*. The second chapter schedules the author's experiments, carried out during four years at Onderstepoort and, in the third chapter, these are discussed. This section is of considerable importance and serves to emphasise the urgent need for the carrying out of large scale and accurately controlled experiments for the elucidation of these problems. There is here a first class field of work for valuable collaboration between veterinarian and mycologist.

Section five, containing some two-thirds of the volume, is devoted to a systematic consideration of poisonous plants, only those which have experimentally been proved to be poisonous being mentioned. Algal poisoning still seems doubtful. It is interesting that the only poisonous fern is the common bracken and the author describes *Equisetum ramosissimum* as the only poisonous horsetail although there is evidence that in Europe many other species may also be noxious. The sole poisonous representative of the Gymnosperms is the Yew. The Phanerogamic natural orders are then worked through, the several species being considered according to the schedule: Taxonomy; active principle; toxicity of the plant; symptoms of poisoning; post-mortem appearances and histology; diagnosis; treatment and prevention. There is little one can say of this part of the book: it all seems to be very thoroughly and accurately done; it is full of the author's own observations and experimental results and, where gaps in knowledge exist, attention is drawn to them. Most of the discussions of individual plant species are commendably brief, but the more important poisonous plants, with the conditions to which they give rise, receive extended consideration as in the case of Cotyledon poisoning, "alopecia" due to *Chrysocoma tenuifolia*, "brain staggers" due to *Matricaria nigellaefolia*, and "dunziekte" or "seneciosis" in man and stock due to *Senecio* spp.—which is the best account of this disease available. This portion of the book ends with a 15 page tabulation of plants examined by Dr Steyn with negative results. Specimens of the plants on which the author has carried out his researches are preserved for reference in the Onderstepoort Herbarium and the National Herbarium, Pretoria, and, in addition, many are grown in the Poisonous Plant Garden at Onderstepoort.

There is a bibliography of some 650 references and a first-class index. The volume contains 135 excellent photographs illustrating most of the important poisonous plants and many of the diseased conditions of stock.

As the Rt Hon. J. C. Smuts says in his Foreword, the volume "represents years of research work at the Onderstepoort Laboratories and in the field, and forms without a doubt the most authoritative treatise yet written on this important subject." In spite of its high price it is a volume which should find a place in every botanical, agricultural and veterinary library and the author is to be congratulated upon a very fine achievement.

W. B. BRIERLEY.

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COMPETITION IN SEEDS MIXTURES AT HIGH ELEVATIONS (850 FT.) AT MOELGLOMEN, TALYBONT, CARDIGANSHIRE

BY M. T. THOMAS, B.Sc.

(*Welsh Plant Breeding Station, Aberystwyth.*)

THE experiment discussed in this paper was designed to obtain further information on the seeding down of upland fields of low fertility. As the rotation on hill farms is usually a long one, to be fully successful the seeds mixture employed must (*a*) provide adequate grazing in the spring for several years, (*b*) form a dense sward quickly to keep out weeds, and (*c*) provide hay crops in the first and second harvest years and frequently in the third year also. As a rule such fields are not closed for hay until at any rate early in May because of the demands made by sheep, in particular at and soon after lambing time. The field, 850 ft. above sea-level, on which the experiment was laid down was representative of enclosed hill land adjacent to the *ffridd*[1]. The soil was thin, light and very stony. Various species and strains of legumes with different strains of perennial rye-grass and cocksfoot were compared, in conjunction with five species of bottom grasses. The seed rates per acre (40–45 lb.) were decidedly higher than are usual, previous experiments having indicated that the prevailing rates are too low, especially in regard to bottom grasses.

SOWING AND SUBSEQUENT TREATMENT.

The seeds were broadcast under oats in May 1930. Hay was taken in each of the three harvest years under consideration. The hay from the several plots was weighed in the first and second harvest years, but not in the third harvest year.

In December 1930 (seeding year), in order to compare the influence of very light periodic grazing with very heavy and more or less continuous spring grazing on the weight of hay and on the formation of the resultant sward, two replications (from a total of five) of each mixture in the test were fenced off and the grazing of these plots controlled. The fence was removed in December 1932 (second harvest year), and since then all the plots have been open to the hard grazing to which the field as a whole was subjected. The purpose of the fence was to protect the seedlings during the critical period in the formation of the sward, especially during the winter of the seeding year, and ultimately to find out the effect of this method of treatment on the botanical composition and productivity of the herbage after a period of years.

---

[1] Enclosed but uncultivated hill land adjoining open mountain.

MATERIAL AND METHODS.

All the plots were 1/100th acre in size and approximately twice as long as broad.

In connection with all the hay weights taken, a 1/500th acre wooden frame (outside measurement) was used. Immediately after cutting, the herbage per plot was weighed by means of a spring balance fixed to a tripod. One 2 lb. representative sample was taken and placed in a scrim bag. The herbage in the scrim bags was dried on poles out in the open. Periodic shaking prevented mouldiness and assisted the drying process. When the sample had been "air-dried" to a constant weight, the weight was recorded and considered as the hay weight. The weight of hay per quadrat (1/500th acre) was then calculated from the green weight, and for convenience the figures given in the tables have been expressed as yields per acre. A further representative sample of approximately 1 lb. was taken for botanical analysis, using the percentage productivity estimation method as normally adopted at the Station for hay samples (1).

Table I.

The seeds mixture used (*in lb. per acre*).

	Mixtures										
Species and strain	1	2	3	4	5	6	7	8	9	10	11
Italian rye-grass	4	4	4	4	4	4	4	4	4	4	4
Cocksfoot (pedigree)*	16	—	—	16	—	16	—	—	—	16	—
Cocksfoot (Danish)	—	16	—	—	16	—	16	—	—	—	16
Cocksfoot (New Zealand)	—	—	16	—	—	—	—	—	—	—	—
Perennial rye-grass (pedigree)*	—	—	—	—	—	—	—	16	—	—	—
Perennial rye-grass (commercial)	—	—	—	—	—	—	—	—	16	—	—
Timothy (pedigree)*	—	—	—	—	—	—	—	10	10	10	10
Crested dogstail	6	6	6	5	5	12	—	6	6	—	—
Rough-stalked meadow grass	—	—	—	6	6	—	12	—	—	—	—
Red fescue (pedigree)*	10	—	—	5	—	—	—	—	—	—	—
Chewing's fescue	—	10	10	—	5	4	4	—	—	—	—
Smooth-stalked meadow grass	—	—	—	—	—	—	—	—	—	6	6
Red clover (Montgomery)	8	8	8	—	—	—	—	—	—	—	—
Lotus major	—	—	—	8	8	8	8	—	—	—	—
Red clover (wild)	—	—	—	—	—	—	—	8	8	—	—
Kidney vetch	—	—	—	—	—	—	—	—	—	8	8
Wild white clover (English)	2	2	2	2	2	2	2	2	2	2	2

* Welsh Plant Breeding Station productions.

Particulars of the mixtures used in the trial are given in Table I. Cocksfoot and perennial rye-grass in all the mixtures in which they are included were sown at the rate of 16 lb. per acre, the legumes (except white clover) at 8 lb. per acre. The bottom grasses were used in various combinations, but in most of the mixtures they totalled 16 lb. per acre.

DISCUSSION OF RESULTS.

First and second harvest years. The schedule given in Table I will show that the various mixtures can be grouped so that many of the data can be analysed on the basis of the average for a number of plots, and thus added significance is given to the fundamental indications in the results obtained. Where necessary the two systems of management have been dealt with separately.

Comparisons of air-dry yields of hay in the first and second harvest years (1931 and 1932) are given in Table II. Yields are affected largely by season, date of cutting and time of "putting up" to hay.

Table II.

To show the total air-dry yields (including sown and unsown species) for the various mixtures in the first and second harvest years respectively. Figures calculated to cwt. per acre (1 *cwt.* = 100 *lb.*).

Mixture		Yields		
		1931	1932	Total for the 2 years
Replicated five times:				
Chiefly cocksfoot and Montgomery red clover and bottom grasses	(1)	33·34	33·45	66·79
	(2)	30·00	32·31	62·31
	(3)	34·08	37·16	71·24
Chiefly cocksfoot and *Lotus* and bottom grasses	(4)	28·92	29·88	58·80
	(5)	26·14	30·22	56·36
	(6)	25·49	28·28	53·77
	(7)	23·58	29·04	52·62
Replicated three times:				
Chiefly perennial rye-grass, timothy and wild red clover and bottom grasses	(8)	34·04	32·93	66·97
	(9)	30·85	34·30	65·15
Chiefly cocksfoot, timothy, kidney vetch and bottom grasses	(10)	21·30	27·02	48·32
	(11)	21·32	27·57	48·89
Average		28·10	31·11	59·21
Relative		100·0	111·0	—

In this trial the hay was "put up" early in May and cut early in July in each year. The increased yield in the second harvest year is significant and is to be attributed very largely to crested dogstail and cocksfoot, both of which have given a much heavier yield than in the first harvest year. In fact, crested dogstail in all the mixtures in which it is included becomes a definite aggressor in the second harvest year, and this is a fact definitely to be reckoned with on fields always "put up" to hay late in the season.

In Table III comparative yields of hay under very light and very hard methods of spring grazing are given.

Table III.

To show comparative yields of hay after "very light" and "very hard" spring grazing. Average of all mixtures. Figures calculated to cwt. per acre (1 *cwt.* = 100 *lb.*).

	1931		1932		Total for the 2 years	
Type of spring grazing ...	Light	Heavy	Light	Heavy	Light	Heavy
Yield	35·22	23·99	35·15	27·94	70·37	51·93
Relative	100	68	100	80	100	74

The figures represent the average of all the mixtures tested and therefore of a very large number of replications for each system of management. The last grazing of the spring on the lightly grazed plots was very light, so as not to retard unduly recovery when the plots were put up to hay. These plots, therefore, started off with a distinct advantage compared to the hard-grazed plots.

The differential grazing has had a marked effect on total yield in both the first and second harvest years. Each mixture has shown the same type of general result, and it is clearly demonstrated that May is too late for "putting up" to hay if high yields are the only consideration, and this is particularly so when the spring grazing is very hard.

In Table IV the yields of pedigree and Danish cocksfoot are compared. Both strains of cocksfoot, even under these conditions and when dominant ingredients in the mixtures, are essentially second-year hay plants. Their sensitivity to competition is well shown in both years, and they have reacted in a very similar manner depending on the other components of the mixture.

Table IV.

To show comparative yields of pedigree and Danish cocksfoot during the first two harvest years. Weight of stem and leaf given separately for the second harvest year. Yields calculated to cwt. per acre (1 *cwt.* = 100 *lb.*).

Pedigree cocksfoot					Danish cocksfoot				
	1931	1932				1931	1932		
Mixture	Stem + leaf	Stem	Leaf	Total	Mixture	Stem + leaf	Stem	Leaf	Total
1	3·27	2·11	3·52	5·63	2	1·32	2·10	1·20	3·30
4	3·40	3·97	5·06	9·03	5	2·96	2·15	1·09	3·24
10	6·12	6·08	7·66	13·74	11	6·18	6·16	2·43	8·59
Average	4·26	4·05	5·41	9·46	Average	3·49	3·47	1·57	5·04

Montgomery red clover and crested dogstail have had a marked depressant effect on the yield of cocksfoot in both years—this is well shown when mixtures 1 and 2 are compared to mixtures 10 and 11. It will be remembered that mixtures 10 and 11 contained kidney vetch and smooth-stalked meadow grass, neither of which contributed appreciably to the herbage at any time of the year. The cocksfoots accordingly had to suffer very little from competition, and their yield increased. Danish cocksfoot has contributed far less than pedigree cocksfoot in both harvest years. The increased yield of pedigree cocksfoot in the second harvest year, even under intense competition, ranks it as a very useful grass for these conditions, and especially under the systems of management to which these swards are usually subjected. Its higher ratio of leaf to stem compared to that of Danish cocksfoot is another important factor, and in keeping with all previous work carried out at the Station (2).

In Table V total yields of pedigree and commercial perennial rye-grass are compared.

Table V.

To show comparative yields of pedigree and commercial perennial rye-grass during the first two harvest years. Yield calculated to cwt. per acre (1 *cwt.* = 100 *lb.*).

	Pedigree			Commercial	
Mixture	1931	1932	Mixture	1931	1932
8	8·82	6·48	9	9·35	2·64

Whereas in cocksfoot the second harvest year yield is higher than that in the first harvest year, with the rye-grass the reverse is the case. In the first harvest year, although competing with wild red clover, the rye-grasses gave a higher yield than cocksfoot, even on the kidney vetch plots where competition was at a minimum. In the second harvest year the pedigree rye-grass shows an appreciable drop, and the commercial rye-grass a very pronounced drop in yield. Unfortunately there were only two mixtures containing perennial rye-grass, and the figures, therefore, represent an average of five replicates only. It is significant that at 6 lb. per acre crested dogstail is an aggressor relative to rye-grass in the second harvest year.

Stem and leaf data are not available for the rye-grasses. In Table VI the effect of differential management (hard and light spring grazing) on the percentage stem to leaf in pedigree and Danish cocksfoot is given.

Table VI.

To show the effect of "very light" compared to "very hard" spring grazing on the percentage stem and leaf in cocksfoot hay in the second harvest year. Percentage productivity estimation.

		Hard spring grazing			Light spring grazing		
Mixture	Strain	Stem %	Leaf %	Total	Stem %	Leaf %	Total
1	Pedigree	8·00	9·33	17·33	4·75	14·00	18·75
2	Danish	6·25	3·88	10·13	7·50	3·00	10·50
4	Pedigree	17·00	16·50	33·50	8·00	17·75	25·75
5	Danish	7·34	2·66	10·00	6·75	5·00	11·75
10	Pedigree	23·33	26·67	50·00	21·25	31·00	52·25
11	Danish	26·67	8·67	35·34	16·00	8·50	24·50
Average	Pedigree	16·11	17·50	33·61	11·33	20·92	32·25
	Danish	13·42	5·07	18·49	10·08	5·50	15·58

The figures are definitely in favour of light grazing for production of leaf in the hay, although they are also influenced by the late date of "putting up" to hay. Hard spring grazing followed by "putting up" later tends to force stem formation at the expense of leaf. Relatively the Danish cocksfoot has suffered more than the pedigree cocksfoot in this respect, and this fact tends to emphasise the need for leafy and heavy tillering strains for these conditions, especially as these two qualities are associated with persistency. Leaf from the nutritive point of view is also vastly superior to stem (3), and any system of management, therefore, which tends to increase percentage leaf should be encouraged.

In Table VII the legumes are compared. Although *Lotus major* makes a fair contribution in the first harvest year its productivity is negligible in the second year.

Table VII.

To show the comparative yields of Montgomery and wild red clovers, Lotus major *and kidney vetch in the first two years. Figures calculated to cwt. per acre* (1 *cwt.* = 100 *lb.*).

	Montgomery red clover	*Lotus major*	Kidney vetch	Wild red clover
1931	19·07 (100)	6·10 (32)	0·39 (2)	10·53 (55)
1932	12·57 (100)	0·68 (6)	0·04 (trace)	6·03 (48)

Lotus major is a very palatable species and does not withstand hard grazing. Other trials have indicated that unless improved strains can be selected *Lotus* is very much inferior to Montgomery red for these conditions. Kidney vetch has been a significant failure in both harvest years. Observations made in the autumn of the seeding year indicated that early establishment was fairly good. It, however, over-wintered very badly, largely as a result of the severity of the grazing, but partly because it would not appear to be suited to the soil conditions. Montgomery red clover gave a heavy yield in both years. Wild red clover came second to Montgomery red clover, but observations made and data collected in the third harvest year suggest that the particular strain employed did not persist any better than Montgomery red clover. Extensive trials carried out by Williams (4) commend the late clovers rather than the early-flowering clovers, especially under poor conditions, since they are more productive even in the first harvest year. They also can withstand hard grazing better. The late "putting up" also gives an added advantage to the Montgomery clover relative to other species. More than 50 per cent. of the first hay crop was contributed by Montgomery red clover, and over 30 per cent. in the second year.

In Table VIII is given the productivity contribution of the various bottom grasses tested in the mixtures.

Table VIII.

To show the yields of the bottom grasses in the various mixtures in the first two harvest years as hay. Figures calculated to cwt. per acre (1 *cwt.* = 100 *lb.*).

	Crested dogstail		Rough-stalked meadow grass		Smooth-stalked meadow grass		Fine-leaved fescues	
Mixture	1931	1932	1931	1932	1931	1932	1931	1932
1 and 2	4·70	9·91	—	—	—	—	1·49	1·67
4 and 5	6·30	11·16	2·41	0·69	—	—	0·75	1·21
6	13·54	15·58	—	—	—	—	0·10	1·53
7	—	—	6·18	2·09	—	—	0·66	4·50
8 and 9	6·75	13·87	—	—	—	—	—	—
10 and 11	—	—	—	—	0·01	0·02	—	—
Average	7·82	12·63	4·30	1·39	0·01	0·02	0·75	2·23
Two years' total relative	100		27		Trace		14	

Crested dogstail is a definite aggressor, and even when sown at 5–6 lb. per acre has outyielded both cocksfoot and perennial rye-grass in the second year. Its contribution to the hay, however, is stemmy and fibrous, and in this respect it is distinctly inferior to both cocksfoot and rye-grass, and particularly to the indigenous strains of these latter species. Under the hard-grazing conditions it has, however, formed an excellent bottom to the sward. In the aftermath of the third harvest year it has been superseded by both the fine-leaved fescues included in the trial. Since pedigree red fescue and Chewing's fescue have behaved very similarly throughout the trial their figures are here averaged. The red fescue in distinct contrast to Chewing's was, however, green right through the winter. Red fescue and Chewing's fescue did not contribute much to the first and second harvest year hay.

Smooth-stalked meadow grass has not yet made any significant contribution to the herbage. Rough-stalked meadow grass has shown a distinct falling off in the second year, and at no time has it contributed as much as crested dogstail at similar seed rates per acre. In mixture 6, crested dogstail, and in mixture 7, rough-stalked meadow grass, were sown respectively at 12 lb. per acre. On all the plots the yield of dogstail was higher than that of the meadow grass in the second harvest year.

In Table IX the average yields of each species and strain in the test are given for the first and second harvest years. The data have been obtained from all the plots and therefore from different mixtures.

Table IX.

To show the yield of each sown species and strain, also weeds, in the first and second harvest years. Average of all mixtures where the various components appear. Yields in cwt. per acre (1 *cwt.* = 100 *lb.*).

	Species and/or strain							
Year	Italian rye-grass	Pedigree cocks-foot	Danish cocks-foot	Pedigree peren-nial rye-grass	Com-mercial perennial rye-grass	Pedigree timothy	Crested dogs-tail	Rough-stalked meadow grass
1931	1·38	3·52	3·39	8·82	9·35	1·71	6·64	3·67
1932	0·02	8·31	5·35	6·48	2·64	2·20	12·08	1·16

	Species and/or strain							
Year	Smooth-stalked meadow grass	Fine-leaved fescue	Mont-gomery red clover	*Lotus major*	Wild red clover	Kidney vetch	Wild white clover	Weeds
1931	0·01	0·59	20·93	5·90	10·53	0·39	1·10	4·57
1932	0·02	2·81	14·08	0·67	6·03	0·04	3·81	4·29

In the first harvest year Montgomery red clover is the outstanding yielder of hay, and the strains of perennial rye are best amongst the grasses. In the second year Montgomery red clover maintains its position but perennial rye-grass does not contribute nearly as much as dogstail, which is highest amongst the grasses. Perennial rye-grass, rough-stalked meadow grass and the legumes (except white clover) have all decreased in yield, commercial rye-grass showing a very significant fall. The cocksfoot, dogstail and fine-leaved fescues and wild white clover, and to a lesser extent pedigree timothy, have each increased in yield, and have helped to maintain or augment productivity in the second year. This is important, as generally there is a marked fall in yield between the first and second years when ordinary commercial mixtures without bottom grasses are used.

Under the poor conditions of the present trial the size of tiller in cocksfoot is very much larger than in rye-grass, the contrast being much more in favour of cocksfoot than under conditions of high fertility. The tendency with perennial rye-grass, a high fertility-demander, is to become dwarfed. Whereas tiller formation in rye-grass is very rapid, in cocksfoot it is comparatively slow, and this accounts to some extent for the increased yield of cocksfoot in the second year.

The position taken by wild white clover as a hay plant in the second harvest year is significant. It has made a higher contribution to the hay than has commercial perennial rye-grass, pedigree timothy, rough-stalked meadow grass or the fine-leaved fescues. This ability of wild white clover to make a definite contribution to the hay crop on poor fields which are "put up" to hay late is probably not sufficiently realised, and adds emphasis to the supreme importance of this clover as an ingredient in swards under poor conditions—it makes a material contribution to bulk, let alone all question of its high protein and calcium content and its influence on soil fertility.

Third harvest year results. No hay data are available for the third harvest year, but detailed analyses taken on the plots in the September aftermath of that year give a useful picture of the trend of events. The figures are given in Table X.

Table X.

To show the percentage contribution by tiller estimations of all the species and/or strains in September of the third harvest year.

Species and strain	1	2	3	4	5	6	7	8	9	10	11
Italian rye-grass	—	—	—	—	—	—	—	—	—	—	—
Pedigree cocksfoot	26·0	—	—	25·5	—	31·0	—	—	—	64·5	—
Danish cocksfoot	—	9·0	—	—	8·5	—	9·0	—	—	—	33·5
New Zealand cocksfoot	—	—	14·0	—	—	—	—	—	—	—	—
Pedigree perennial rye-grass	—	—	—	—	—	—	—	41·5	—	—	—
Commercial perennial rye-grass	—	—	—	—	—	—	—	—	31·0	—	—
Pedigree timothy	—	—	—	—	—	—	—	5·5	10·0	7·0	9·0
Crested dogstail	4·0	6·0	5·0	5·0	7·0	8·0	—	13·0	13·0	—	—
Rough-stalked meadow grass	—	—	—	3·5	4·0	—	5·0	—	—	—	—
Smooth-stalked meadow grass	—	—	—	—	—	—	—	—	—	4·0	7·0
Red fescue (pedigree)	61·0	—	—	54·5	—	—	—	—	—	—	—
Chewing's fescue	—	68·5	69·0	—	65·5	48·0	67·0	—	—	—	—
Red clover (Montgomery)	0·5	1·0	1·0	—	—	—	—	—	—	—	—
Lotus major	—	—	—	Trace	Trace	0·5	Trace	—	—	—	—
Red clover (wild)	—	—	—	—	—	—	—	0·5	0·5	—	—
Kidney vetch	—	—	—	—	—	—	—	—	—	Trace	—
Wild white clover	3·0	3·5	4·5	6·0	7·0	6·5	8·0	10·0	12·0	11·5	15·5
Weeds	5·5	12·0	6·5	5·5	8·0	6·5	11·0	29·5	33·5	13·0	35·5

Both the fine-leaved fescues (pedigree red fescue and Chewing's fescue) became definite aggressors in the third year on those plots where they were sown, while on the other plots their contribution lacked significance. Previous work on lowlands in regard to Chewing's fescue at heavy seed rates per acre has given similar results (5). Under conditions on Welsh hill farms of the type under discussion sward reversion is common by way of dominance in self-regenerating Yorkshire fog, bent and fine-leaved fescue. The data show that in the present experiment very little fine-leaved fescue had made an unsown appearance by the third year, but that where sown even at 4 lb. per acre it had become highly aggressive.

Crested dogstail at this time was not a major contributor on the plots. Similarly, cocksfoot on the basis of tiller counts does not show up favourably, but the large size of tiller gives it a very definite place as a producer of aftermath under these conditions. It will be noticed that pedigree cocksfoot is greatly superior to Danish cocksfoot in all the mixtures; New Zealand cocksfoot is intermediate, but as there is only one mixture in the trial containing this strain the data can only be regarded as indicative; still it is of interest to note that the data are wholly in keeping with previous results obtained at the Station.

In all mixtures containing red or Chewing's fescue the percentage tillers of other species and weeds is low because the total number of tillers is high, due to the smallness and abundance per unit area of the tillers of these fine-leaved fescues. The tillers of wild white clover and weeds are high in mixtures 8, 9, 10 and 11, partly because the total tillers are about half as numerous as in the other mixtures. There are, however, indications from the figures that Montgomery red clover with the fine-leaved fescues and crested dogstail have had a depressant effect on wild white clover. It is also highly probable that the fine-leaved fescues forming a dense sward in the third year have helped to keep the plots comparatively free from weeds and unsown grasses. Those mixtures which do not contain these strains have the highest percentage of unsown species. There is evidence to show that pedigree cocksfoot is much more effective in delaying reversion than is Danish cocksfoot, and this is so even when dogstail and the fine-leaved fescues are not included in the mixture; and in this respect it would appear to be definitely superior to pedigree rye-grass (compare mixtures 8, 9, 10 and 11).

The data show that both red and Chewing's fescue spread rapidly once they become firmly established.

In Table XI counts (actual counts in these cases) of tillers of pedigree and Danish cocksfoot, pedigree and commercial perennial rye-grass and pedigree timothy in the third harvest year are given.

Table XI.

To show the number of tillers per $2\frac{1}{2}$ sq. ft. of perennial rye-grass, cocksfoot and timothy in the aftermath of the third harvest year.

Mixture	Pedigree cocksfoot	Danish cocksfoot	New Zealand cocksfoot	Pedigree perennial rye-grass	Commercial perennial rye-grass	Pedigree timothy
1	329	—	—	—	—	—
2	—	116	—	—	—	—
3	—	—	178	—	—	—
4	295	—	—	—	—	—
5	—	107	—	—	—	—
6	312	—	—	—	—	—
7	—	131	—	—	—	—
8	—	—	—	333	—	50
9	—	—	—	—	282	83
10	423	—	—	—	—	43
11	—	159	—	—	—	51

It will be noticed that the effect of initial competition and also the aggressiveness of the fine-leaved fescues affect the number of tillers of cocksfoot per unit area (mixtures 10 and 11 were devoid of fine-leaved fescue). Pedigree cocksfoot and pedigree perennial rye-grass compare quite favourably in regard to the number of tillers per $2\frac{1}{2}$ sq. ft.; the size of tiller, however, is vastly different and very markedly in favour of cocksfoot. In both species the pedigree strains show to marked advantage over the non-pedigree. The highly palatable pedigree timothy has been unable to persist in telling amount.

PRACTICAL CONCLUSIONS AS TO SEEDS MIXTURES.

The evidence from the observations and data discussed in this paper indicates that competition as a factor in pasture establishment is just as important at high elevations as on the lowlands, although operating to a large extent in a very different manner. The range of useful species is, however, more limited. Rye-grass and rough-stalked meadow grass to be really productive are definitely high fertility-demanders, and the evidence points to pedigree cocksfoot and crested dogstail as very much superior under poor conditions. Rough-stalked meadow grass does not show to advantage on these uplands and cannot compare to crested dogstail even if heavy seed rates are used. In the second year crested dogstail, although competing with pedigree cocksfoot, gave a heavier yield than rough-stalked meadow grass when the latter had only to compete with Danish cocksfoot. Comparisons of tiller counts in the third harvest year show that Montgomery red clover and crested dogstail have been aggressors compared to cocksfoot. Where cocksfoot was sown without dogstail and Montgomery red clover it covers more ground and provides a greater number of tillers per unit area than where sown in competition with these species. The data indicate in this connection that dogstail has been even more aggressive than the red clovers. The fine-leaved fescues are also highly aggressive by the aftermath of the third year, even though the individual tiller of cocksfoot in comparison is much larger. Previous trials have shown Montgomery red clover to be a superior strain to all the other red clovers under upland conditions generally. Ordinary *Lotus major* has to all intents and purposes proved a failure in this trial. There are reasons for supposing that improved strains of this species may be found useful on certain of our hill pastures. It is probable, however, that it will always be safer to rely on Montgomery red clover as the most suitable legume for hay and grazing under upland conditions in general. It is also probable that when seeding down hill country for hay and grazing pedigree cocksfoot should be the dominant ingredient in any mixture, and this should be supplemented by a relatively light seeding of perennial rye-grass to prevent undue grazing of the cocksfoot in the first year.

Crested dogstail and pedigree red fescue in appreciable amount (3–4 lb. of the former and 2 lb. of the latter per acre) are essential in order to form a good sward and will also contribute in large measure to the hay. Red fescue has distinct advantages over Chewing's fescue, because it is more palatable and does not get winter-burned. Smooth-stalked meadow grass is not suited to the conditions.

Wild white clover should always be included on the score no less of bulk than of nutritive value, soil fertility and as an influence suppressing weeds. Italian rye-grass is essential for spring keep, and the indications are that the rate per acre for poor fields at high elevations where the hay will be "put up" late should be much higher than has hitherto been supposed—it should be up to 10 or 12 lb. per acre. Pedigree timothy, as was to have been expected, has not done well on this light soil on which the trial was conducted; on heavier and peaty soils there are indications, however, that it may prove successful even on poor fields at high elevations.

SUMMARY.

The experiment was designed to obtain further information on the value of certain species and strains of grasses and legumes for permanent grass on upland, thin soiled, dry fields of low fertility.

Their ability to compete in mixtures and their persistency were studied in detail and the evidence indicates, in a general way, that these

factors operate in rather a different manner on the uplands as compared to the lowlands.

Pedigree cocksfoot is superior in productivity to pedigree perennial rye-grass, but the need for a small seeding of pedigree perennial rye-grass to help protect the pedigree cocksfoot during the first few years is suggested.

Montgomery red clover has proved itself to be infinitely better than *Lotus major*, kidney vetch and wild red clover, especially for hay. Of the bottom grasses in the test, smooth-stalked meadow grass has been a failure, and although rough-stalked meadow grass made a fair contribution to the second crop of hay when sown at a heavy seed rate, crested dogstail and the fine-leaved fescues have been far more aggressive. The heavy contribution of crested dogstail to the second-year hay crop is worthy of note. The aggressiveness of the fine-leaved fescues in the aftermath of the third harvest year is a factor to be further considered. Chewing's fescue, in distinct contrast to red fescue (pedigree), gets very winter-burned and unpalatable.

Wild white clover is an essential constituent in mixtures for these conditions. It is probable that Italian rye-grass at 10 or even 12 lb. per acre is not too much where the spring grazing is hard and where the fields are not closed for hay until early May. Indigenous and pedigree strains have given more hay, more leaf and have been more persistent than the commercial strains.

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A COMPARISON OF ENGLISH AND CANADIAN TOMATO VIRUS DISEASES

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(With Plates XXVI and XXVII.)

Introduction.

In recent years a considerable literature dealing with tomato virus diseases, especially those of the "streak" type, has been published both in America and in this country, but as no comparative study of the diseases had been made considerable doubt existed whether certain diseases with a common symptom picture, which occur in both countries, were in reality identical from a causal standpoint. Still further confusion had resulted from the fact that tomato streak has been found in England to be caused, in the majority of cases, by a single virus, whereas in America a disease with identical symptoms was considered to be caused by a mixture of a potato and a tobacco virus. The whole question, therefore, of the virus diseases of tomato seemed to need clarifying, and it was with this idea in mind that the present comparative study was started in July 1933, when it was possible to have the collaboration of G. H. Berkeley who was on a year's visit to this country. The work here recorded has been carried out by the three authors in their respective laboratories and notes have been compared from time to time. In this way it was hoped to eliminate errors due to any accidental infections, and the conclusions, while agreed upon by the three authors in collaboration, have been arrived at by each independently. It was hoped that such a study would enable us to define certain viruses which are known to attack the tomato and to associate them with the diseases found in that crop both in England and in Canada. As a result of this study it has been found that the more common viruses occur in both countries.

Nomenclature.

It is probably desirable at this point to make clear the distinction between a virus and a virus disease. In the host plant a group of abnormal symptoms characterise a disease. The symptoms may be caused by any pathogen or group of pathogens and these in turn may be viruses, if they conform to the recognised requirements. A single *virus* may be responsible for more than one *disease*, *e.g. tobacco virus No.* 1 causes tobacco mosaic in tobacco and tomato mosaic in tomato. A single disease condition, on the other hand, may be caused by different viruses, *e.g.* tomato streak, which may be caused by a single virus or by a mixture of two viruses.

Reference must be made to a paper by Johnson (21) in which he described the properties of nine different tobacco viruses. These viruses, he was able to show, caused distinct diseases in tobacco and had various characteristics which separated them one from another. In this paper Johnson's nomenclature has been employed for the viruses which he described, in so far as they appeared in our material. Other viruses which have been found have been defined as precisely as possible with the view to clarifying the confusion which indisputably exists, not only in the earlier but also in the more recent literature.

In the first part of this paper the tomato mosaics, the streak complex and spotted wilt are defined, the literature cited and the characteristics of the viruses described. In the second part, a detailed description of the results obtained from the examination and comparison of the English and Canadian material is given, together with a discussion of the general problem.

Part I. Description of the diseases and the viruses.

(1) *Tomato mosaic.*

Disease synonyms. Ordinary or mild tomato mosaic.

Virus. *Tobacco virus No.* 1 (Johnson (21)).

Literature. Tobacco mosaic was the first plant disease caused by a filterable virus to be recognised (Ivanowski, 1892), and Clinton in 1908 first demonstrated that tobacco mosaic could be transmitted to tomato and *vice versa* (see Burnett and Jones (12)). Common tobacco mosaic is caused by Johnson's *tobacco virus No.* 1 (21), the type disease being that described by Allard (1). Allard (5) considered the viruses of tobacco mosaic and of tomato mosaic to be identical, and this view has been generally held by later workers (see Dickson (13), Walker (32), Burnett and Jones (12), Ainsworth (2) and others).

DISEASE SYMPTOMS. *Tomato.* Dark green—light green mottle (see Plate XXVI, fig. 2), slight stunting, varying degree of leaf distortion but no necrosis. In winter mottle absent or very slight, but leaf distortion and stunting more severe.

Nicotiana tabacum. No local necrotic lesions, dark green—light green mottle and malformation of leaves. Some stunting and "break" in flower colour.

Nicotiana glutinosa.
Datura Stramonium. } Local necrotic lesions, no systemic infection.

PROPERTIES OF TOBACCO VIRUS NO. 1. *Filterability.* Passes Pasteur-Chamberland filters L1–L7.

Resistance to ageing. Survives *in vitro* for many years.

Resistance to heat. Survives 80° C. for 10 min. Destroyed at 90° C. for 10 min.

(2) *Yellow mosaic of tomato.*

DISEASE SYNONYMS. "Aucuba" mosaic of tomato. This disease is quite generally called "aucuba mosaic" of tomato, but, as the name has in the past led to some confusion with the aucuba mosaic of potato, it is suggested that the name yellow mosaic is preferable.

VIRUS. *Tobacco virus No.* 6 (Johnson (21)).

LITERATURE. This disease was first described in England by Bewley (8) and later in more detail by Henderson Smith (24), who suggested that the virus responsible was the same as that of yellow mosaic of tobacco. Further work has shown that the two viruses are indistinguishable.

DISEASE SYMPTOMS. *Tomato.* Bright yellow mottle on leaves (see Plate XXVI, fig. 3) and fruit (see Plate XXVII, fig. 9), some leaf distortion and stunting of plant. In winter marked leaf distortion and stunting.

N. tabacum. Local necrotic lesions, bright yellow mottle, some necrosis, malformation, stunting and "break" in flower colour.

N. glutinosa.
D. Stramonium. } Local necrotic lesions. No systemic infection.

There is some evidence of the existence of definite strains of this virus. These are being investigated by one of the present authors (J. C.) and will be dealt with in a later paper.

PROPERTIES OF TOBACCO VIRUS NO. 6. *Filterability.* Passes Pasteur-Chamberland filters L1–L7.

Resistance to ageing. Survives several years *in vitro.*

Resistance to heat. Survives 80° C. for 10 min. Destroyed at 90° C. for 10 min.

(3) *Tomato streak.*

LITERATURE. "Streak" disease of tomato was first recorded in America by Lodeman in 1892 and in the same year was described by Bailey as a bacterial disease (see Burnett and Jones (12)). In 1916 streak was attributed by Howitt and Stone (16) to malnutrition, while later (1925) Stone (28) considered the disease to be associated with an excess of nitrogen and a deficiency of potash in the soil and that it could be controlled by increasing the phosphate and potash.

Jackson (17) in 1917 expressed the opinion that streak was a virus disease, and a similar view was held by Gardner and Kendrick (15) who described streak as a form of "tomato mosaic." Dickson (13) attributed streak of Quebec to a double infection with tobacco mosaic and potato mosaic viruses. That streak can be produced in this way has been confirmed by many other American workers including Johnson (20), Berkeley (7), Blood (10), Stover (29), Doolittle and Blood (14), Vanterpool (31), and Burnett and Jones (12), while Valleau and Johnson (30) were able to produce streak in tomato by combining the latent potato virus (see p. 577) with three strains of tobacco mosaic, three strains of etch and three strains of cucumber mosaic. The literature suggests that the tomato streak of America is regarded as being due to a mixed virus infection, though Valleau and Johnson (30) also found a single virus (that of ring mosaic) belonging to the tobacco virus group, which caused streak in tomato, and Johnson (21) described *tobacco virus No.* 9 as the cause of "tomato stem-necrosis," a type of streak.

In England, Paine and Bewley (22) attributed "stripe" disease to *Bacillus lathyri*, and later Bewley and Corbett (9) recognised a connection between "stripe" and "mosaic." Jarrett (18) was of the opinion that tomato streak was due to *tobacco virus No.* 1 without the participation of potato mosaic, but she also recognised the mixed virus condition and distinguished it as "experimental streak." The position was re-examined by Ainsworth (2), who concluded that a mixed-virus streak was of very rare occurrence in the British Isles; a single virus, distinct from *tobacco virus No.* 1, being responsible for the disease, which, following Jarrett's terminology, he called *glasshouse streak*. A possible explanation of the discrepancies between the results of Ainsworth (2) and those of Jarrett (18) and Bewley and Corbett (9) is that the earlier workers used mixtures of tomato mosaic and single-virus streak, for such mixtures (as pointed out (2), p. 425) may remain long undetected if serial transfers are made through tomato.

K. M. Smith (27) in his recent survey of the plant viruses described single-virus streak as a type of "mosaic" and suggested that streak in glasshouses was caused by double infection with spotted wilt and tomato mosaic viruses. This latter view is untenable as streak is prevalent on commercial nurseries from which spotted wilt is known to be absent. We think that K. M. Smith has not attached sufficient importance to the existence of a single virus which causes the greater part of the streak under commercial conditions.

NOMENCLATURE. From the above it is at once apparent that there is more than one "streak" disease of tomato, and as a result of this investigation it is proposed to recognise at the present time the four following streak diseases. Although in so far as symptoms are concerned these diseases may be identical, nevertheless they have been proved to be caused by different viruses. They are:

(i) Single-virus streak;
(ii) Mixed-virus streak;
(iii) Stem-necrosis streak;
(iv) Ring mosaic streak.

(i) *Single-virus streak.*

DISEASE SYNONYMS. Glasshouse streak or "stripe." We suggest that both these synonyms be dropped. The first because this disease is a trouble of outdoor as well as of glasshouse tomatoes and the second because "stripe," which was first used by Bewley in connection with a bacterial disease exhibiting streak-like symptoms, should be used only in the original sense, that is, in connection with *Bacillus lathyri*, as the cause of stripe disease.

VIRUS. *Tomato streak virus No.* 1 (this paper).

DISEASE SYMPTOMS. *Tomato.* Necrotic lesions on stem, leaves and fruit, mottle, some leaf distortion and stunting of plant (see Plate XXVII, figs. 5 and 6). Under certain conditions the disease may manifest itself as a mottle only.

N. tabacum. Local necrotic lesions—no other symptoms or systemic necrosis, mottle and stunting.

N. glutinosa. }
D. Stramonium. } Local necrotic lesions, no systemic infection.

PROPERTIES OF TOMATO STREAK VIRUS No. 1. *Filterability.* Passes Pasteur-Chamberland filters L 1–L 7.

Resistance to ageing. Survives *in vitro* several years.

Resistance to heat. Survives 80° C. for 10 min. Destroyed by 90° C. for 10 min.

(ii) *Mixed-virus streak.*

DISEASE SYNONYMS. Experimental streak, winter blight.

VIRUS. A mixture of two or more viruses. Generally this mixture is a tobacco mosaic virus or *tomato streak virus No.* 1, and a potato virus of the *X* type (*Potato virus X*, see Smith (25)).

DISEASE SYMPTOMS. *Tomato.* Necrotic lesions on stem, leaves and fruit, mottle, some leaf distortion and stunting of plant. A mottle without any necrosis is rare (cf. single-virus streak above).

N. glutinosa. } Local lesions followed by systemic symptoms
D. Stramonium. } caused by the potato virus fraction.

These host plants are useful indicators for streaks of the mixed-virus type, since the potato fraction of such mixtures produces systemic symptoms in them.

PROPERTIES OF VIRUSES OF THE TOBACCO MOSAIC GROUP. See above.

PROPERTIES OF POTATO MOSAIC VIRUS (*X* TYPE). *Filterability.* Passes L3 but not usually L5 or L7 Pasteur-Chamberland filters.

Resistance to ageing. Survives *in vitro* six months or less.

Resistance to heat. Survives 60° C. for 10 min. Destroyed by 70° C. for 10 min.

(iii) *Stem-necrosis streak*, see Part II, p. 576.

(iv) *Ring mosaic streak*, see Part II, p. 576.

(4) *Spotted wilt of tomato.*

VIRUS. *Spotted wilt* or "*T.S.W.*" *virus* (type Samuel *et al.* (23)).

LITERATURE. This disease was first noted by Brittlebank (11) in Australia. The type disease was described by Samuel *et al.* (23), and the description amplified by Bald and Samuel (6). K. M. Smith (26) first detected the disease in England and his findings have been confirmed by Ainsworth (1) and others.

DISEASE SYMPTOMS. *Tomato.* First symptoms a bronzing of the apical leaves (see Plate XXVII, fig. 7) and stunting, later a yellow mottle of the older leaves. Fruit marked (see Plate XXVII, fig. 8). Lesions rarely found in the stem or petioles; cf. streak above.

N. tabacum. Local necrotic lesions, systemic necrosis, stunting.

N. glutinosa. Local necrotic lesions, systemic necrosis, stunting.

D. Stramonium. Local necrotic lesions. Systemic symptoms: mottle, leaf distortion and stunting of plant.

PROPERTIES OF SPOTTED WILT VIRUS. *Filterability.* Cannot be filtered through Pasteur-Chamberland filters.

Resistance to ageing. Survives *in vitro* only a few hours.

Resistance to heat. Destroyed at 45° C. for 10 min.

This virus can attack a large number of different plants and is widely distributed.

Insect vectors.

At the present time no insect vectors of *tobacco virus No.* 1, *tobacco virus No.* 6, *tomato streak virus No.* 1 or *potato virus X* are known.

Spotted wilt virus is very efficiently spread by *Thrips tabaci* Lind. in this country and by the allied insect *Frankliniella insularis* Franklin in Australia (see (26) and (23)).

The preliminary differentiation of the common viruses of the tomato.

It can be seen from the descriptions of the viruses that the five most commonly occurring types can be readily separated from each other by their reactions on two host plants, *N. tabacum* and *N. glutinosa*.

Table I summarises the relevant details for the preliminary differentiation of the viruses of common tobacco and tomato mosaic, potato mosaic, yellow mosaic of tomato, single-virus streak and spotted wilt.

Table I.

Differentiation of the common tomato viruses.

Virus of	No local lesions but systemic chlorosis in *N. tabacum*	Systemic chlorosis in *N. glutinosa*	Local lesions and systemic chlorosis in *N. tabacum*	Local lesions and either no systemic symptoms or systemic necrosis in *N. tabacum*	No symptom after 24 hours' exposure of juice
Tomato mosaic (common tobacco mosaic)	+	–	–	–	–
Potato mosaic	–	+	–	–	–
Yellow mosaic of tomato	–	–	+	–	–
Single-virus streak	–	–	–	+	–
Spotted wilt	–	–	–	–	+

PART II. EXAMINATION OF THE CANADIAN MATERIAL.

The samples of the Canadian material examined were collected by G. C. Chamberlain, Laboratory of Plant Pathology, St Catharines, Ontario, and by W. Newton, Laboratory of Plant Pathology, Saanichton, British Columbia, to whom we tender our best thanks. The material sent was considered by the collectors to be quite typical of the disease condition it represented.

The material was sent to this country in a dried state, and on arrival each sample was divided, portions being independently examined at the

Cheshunt Experimental Station, the East Malling Research Station and Rothamsted Experimental Station. All the samples were examined at two of these stations and most at all three, so that the findings of any one observer were readily confirmed by the others. There was general agreement among the collaborators, and the results presented are, unless stated to the contrary, based on the work of all three authors.

The different viruses found were tested on a range of host plants over a period of nearly a year and were compared with the standard material of this country.

Methods. The method of inoculation was to macerate the diseased tissue in water in a mortar, and to rub the leaves of the test plant gently with a plug of cotton-wool or muslin soaked in the inoculum thus prepared.

As the object of the investigation was to compare English and Canadian tomato virus diseases it seemed advisable to test the viruses concerned on both English and Canadian varieties of the host. Accordingly the common Canadian tomato variety Grand Rapids was used at East Malling, and the English varieties Kondine Red and E.S. 1 at Rothamsted and Cheshunt respectively. White Burley tobacco and a Virginian tobacco and the same strain of *Nicotiana glutinosa* were used by all three investigators as the differential hosts.

The plants were grown in individual pots, inoculated when from 3 to 6 in. high, and were usually kept under observation for a further 4–5 weeks.

All tests of the effects of the viruses on the different host plants were carried out several times at each station in order to obtain the symptom picture under both summer and winter growing conditions.

Experimental results.

Table II summarises the details of the material examined and the viruses isolated. The diseased material, as received, was inoculated into tomato, tobacco and *N. glutinosa* plants and the virus or viruses present identified. Table III summarises the symptoms obtained and other details of the experiments.

The viruses found in the Canadian material.

(*a*) Canadian tomato mosaic.

Four samples (Table II, *a*, *b*, *c*, *k*) labelled tomato mosaic were received, and on examination two (*a* and *c*) proved to be true tomato mosaic (*i.e.* caused by *tobacco virus No.* 1), while the other two (*b* and *k*) were found to contain the virus of single-virus streak (*i.e. tomato streak*

Table II.

List of material with viruses found.

		Viruses obtained
Canadian material:		
a) Tomato mosaic	Vineland, Ontario (tomato var.* Riverside Favourite)	*Tobacco virus No.* 1
(*b*) Tomato mosaic	Stamford, Ontario	*Tomato streak virus No.* 1
(*c*) Tomato mosaic	Vineland, Ontario	*Tobacco virus No.* 1
(*d*) Tomato streak (7 samples)	Stamford, Ontario	*Tomato streak virus No.* 1
(*e*) Tomato streak	Grimsby, Ontario	*Tobacco virus No.* 1
(*f*) Tomato streak	Beamsville, Ontario	*Tomato streak virus No.* 1
(*g*) Tomato streak	St Catharines, Ontario (tomato var. Sutton's Favourite)	*Tobacco virus No.* 1 *Tobacco virus No.* 9
(*h*) Tomato streak	Niagara Falls, Ontario	*Tomato streak virus No.* 1 and potato virus *X* type
(*i*) Tobacco mosaic	Ontario	*Tobacco virus No.* 1
(*j*) Irish Cobbler potato "mosaic"[2]	New Brunswick	Potato virus *X* type
(*k*) Tomato mosaic	British Columbia	*Tomato streak virus No.* 1
(*l*) Yellow tomato mosaic	British Columbia	*Tobacco virus No.* 6
(*m*) Tomato streak	British Columbia	*Tomato streak virus No.* 1 and potato virus *X* type
(*n*) Tomato streak	British Columbia	*Tomato streak virus No.* 1
(*o*) Tomato streak	British Columbia	*Tomato streak virus No.* 1
(*p*) Ring mosaic	Kentucky, U.S.A.	*Ring mosaic virus*
English material:		
(*q*) Tomato mosaic	Cheshunt	*Tobacco virus No.* 1
(*r*) Tobacco mosaic	Rothamsted (authentic material from Johnson)	*Tobacco virus No.* 1
(*s*) Tomato streak	Cheshunt	*Tomato streak virus No.* 1
(*t*) Tomato spotted wilt	Cheshunt	*Spotted wilt virus*
(*u*) Yellow tomato mosaic	Rothamsted (authentic material of Henderson Smith)	*Tobacco virus No.* 6

* All Canadian tomato material was Grand Rapids variety unless stated otherwise.

Table III.

Details of plants inoculated and symptoms.

	Tomato	*N. tabacum*	*N. glutinosa*	Virus
Canadian material:				
(*a*) Tomato mosaic	M. (12)*	M., m. (9)	l.l. (6)	Tb.v.1
(*b*) Tomato mosaic	M. or M. S. (12)	l.l., S. (9)	l.l. (6)	T.st.v.1
(*c*) Tomato mosaic	M. (52)	M., m. (27)	l.l. (14)	Tb.v.1
(*d*) Tomato streak (all samples)	M. or M. S. (63)	l.l., S. (33)	l.l. (14)	T.st.v.1
(*e*) Tomato streak	M. (52)	M., m. (37)	l.l. (17)	Tb.v.1
(*f*) Tomato streak	M. or M. S. (49)	l.l., S. (21)	l.l. (8)	T.st.v.1
(*g*) Tomato streak	M. M. or S. (64)	M., m. M. (mild) (47)	l.l. (14)	Tb.v.1 Tb.v.9
(*h*) Tomato streak	S. (36)	l.l., S. (34)	l.l., M. (17)	T.st.v.1 P.v.*X*
(*i*) Tobacco mosaic	M. (34)	M., m. (25)	l.l. (18)	Tb.v.1
(*j*) Irish Cobbler potato mosaic	M. (mild)† (20)	M. (mild) (7)	M. (6)	P.v.*X*
(*k*) Tomato mosaic	M. or M. S. (23)	l.l., S. (9)	l.l. (10)	T.st.v.1
(*l*) Yellow tomato mosaic	M. (6)	l.l., M., S. (3)	l.l. (2)	Tb.v.6
(*m*) Tomato streak	S. (9)	l.l., S. (5)	l.l., M. (3)	T.st.v.1 P.v.*X*
(*n*) Tomato streak	M. or M. S. (15)	l.l., S. (10)	l.l. (8)	T.st.v.1
(*o*) Tomato streak	M. or M. S. (6)	l.l., S. (4)	l.l. (2)	T.st.v.1
(*p*) Ring mosaic	S. (32)	l.l., S. (18)	l.l. (18)	Ring mosaic virus
English material:				
(*q*) Tomato mosaic	M. (19)	M., m. (9)	l.l. (7)	Tb.v.1
(*r*) Tobacco mosaic	M. (17)	M., m. (8)	l.l. (2)	Tb.v.1
(*s*) Tomato streak	M. or M. S. (19)	l.l., S. (9)	l.l. (6)	T.st.v.1
(*t*) Spotted wilt	Bronzing (27)	l.l., S. (22)	l.l., S. (12)	Spotted wilt virus
(*u*) Yellow tomato mosaic	M. (20)	l.l., M., S. (12)	l.l. (12)	Tb.v.6

M. = mottle without necrosis.
m. = malformation.
S. = streak on tomato, necrosis on tobacco or *N. glutinosa*.
l.l. = local lesions.

Tb.v.1 = *Tobacco virus No.* 1.
Tb.v.6 = *Tobacco virus No.* 6.
Tb.v.9 = *Tobacco virus No.* 9.
T.st.v.1 = *Tomato streak virus No.* 1.
P.v.*X* = potato virus *X* type.

* Numbers within brackets denote total numbers of plants infected to study the symptom pictures.

† Also slight necrosis.

virus No. 1), and they presumably, when collected, showed the mottle symptom only and were therefore considered to be mosaic.

The viruses of the two types were compared with the respective standard strains, *tobacco virus No.* 1 (*r*) and *tomato streak virus No.* 1 (*s*), as regards differential hosts, filterability and resistance to heat, and they were found to be identical with them. For differential host reactions and properties of these viruses see pp. 568 and 570.

(*b*) Canadian yellow mosaic of tomato.

One sample only (*k*), from British Columbia, was examined, and this proved to be identical in all respects with the English material (*u*) when compared at East Malling and Rothamsted. For differential host reactions and properties of this virus (*tobacco virus No.* 6), see p. 568.

(*c*) Canadian tobacco mosaic.

One sample only (*i*) was obtained from Ontario, and on examination the virus proved to be identical in all respects with *tobacco virus No.* 1. For differential host reactions and properties of this virus see p. 568.

(*d*) Canadian tomato streak material.

In all, fourteen samples of tomato streak were obtained of which ten (seven of *d*, *f*, *m* and *o*) proved to be single-virus streak, *i.e.* caused by *tomato streak virus No.* 1. This is the first record of single-virus streak in Canada. For differential host reactions and properties of this virus see p. 570.

Two samples, one from Niagara Falls, Ontario (*h*) and the other from British Columbia (*m*) proved to be mixed-virus streak, the components in both cases being *tomato streak virus No.* 1 and a potato virus of the *X* type. For differential host reactions of properties of these viruses see pp. 570 and 571.

In one sample (*e*), though the diseased material as received showed distinct necrotic lesions on the stem and petioles, only *tobacco virus No.* 1 was detected. The explanation is probably that in this instance the disease was a streak of the mixed-virus type and that the potato virus fraction did not survive the transportation period; otherwise it is difficult to account for the streak lesions on the original material.

One sample from St Catharines (*g*) showed streak symptoms. At Cheshunt and Rothamsted the only virus found in this material was *tobacco virus No.* 1, while at East Malling a virus thought to resemble *tobacco virus No.* 9 (the cause of stem-necrosis of tomato, Johnson (21)) was isolated. This appears to be the first record of the occurrence of *tobacco virus No.* 9, which has not so far been isolated from English material, since Johnson originally defined it in 1927 (21). The differential host reactions and properties of this virus may well be detailed here.

Stem-necrosis streak.

VIRUS. *Tobacco virus No.* 9 (Johnson (21)).

DISEASE SYMPTOMS. *Tomato.* Mottle on leaves with necrosis on petioles and stems.

N. tabacum. No local lesions on rubbed leaves. Faint systemic mottle without stunting or distortion.

N. glutinosa. } Local necrotic lesions. No systemic infection.
D. Stramonium. }

PROPERTIES OF TOBACCO VIRUS NO. 9. *Filterability.* Passes Pasteur-Chamberland filters L1–L7.

Resistance to ageing. Survives 3 months or more (Johnson (21)).

Resistance to heat. Survives 80° C. for 10 min. Destroyed at 90° C. for 10 min.

Ring mosaic streak of tomato.

This disease was not actually found among the plant material from commercial nurseries of England or Canada, but a sample was kindly sent to us by E. M. Johnson of Kentucky. For the first few months the material was examined at Rothamsted and later some was sent to East Malling and Cheshunt. This disease of tomato has been described by Valleau and Johnson (30) and by Johnson (19), but the virus which causes it has not yet been described. The properties and host reactions of the virus have been determined and are appended.

Ring mosaic streak.

VIRUS. *Ring mosaic virus.*

DISEASE SYMPTOMS. *Tomato.* Occasional yellow areas on leaves, with little mottle or distortion. Necrotic lesions irregularly distributed on stem, petioles and leaves of older parts of the plant (see Plate XXVI, fig. 4). Little stunting. Under our conditions we did not get the very severe foliage symptoms described by E. M. Johnson (19) for this virus on tomato but the fruit was seriously affected. Green fruit exhibited chlorotic or necrotic areas and ripe fruit was disfigured by the irregular development of red pigment in the skin.

N. tabacum. Local lesions followed by systemic chlorosis and some necrosis—often ring-like.

N. glutinosa. } Local necrotic lesions, no systemic infection.
D. Stramonium. }

PROPERTIES OF RING MOSAIC VIRUS. *Filterability.* Passes Pasteur-Chamberland filters L1–L7.

Resistance to ageing. Survives some years in dry tissues.

Resistance to heat. Survives 70° C. for 10 min. Destroyed at 80° C. for 10 min.

Irish Cobbler potato mosaic.

Infected Irish Cobbler potato tubers from New Brunswick were sprouted at East Malling and leaves sent to Cheshunt. The virus isolated at both East Malling and Cheshunt was found to be a potato virus of the *X* type.

POTATO VIRUSES.

Burnett and Jones (12) in their investigation of the effects of certain potato viruses on tomato plants distinguished two types of the "latent" potato virus, viz. the "latent" and the "virulent latent." Both these types have been studied during the present work.

From the various samples of Canadian tomato material three isolations of potato viruses were made. Two isolated at Cheshunt approximated to the "latent" virus while one isolated at East Malling was nearer the "virulent latent." In an attempt to establish the identity of these viruses they, together with the Irish Cobbler potato virus, were compared at Cheshunt with authentic *potato virus X*, kindly supplied by K. M. Smith, and several other strains of the *X* virus. As the result of this comparison, a more detailed account of which has been given by Ainsworth (3), it was concluded that all these viruses are potato viruses of the *X* type differing from each other merely in virulence.

These viruses are filterable through L1 and L3 Pasteur-Chamberland filters but not usually through L5 and L7, and are inactivated by heating to 70° C. for 10 min. The symptom picture on tomato varies from a mild or almost imperceptible leaf-mottle to a well-defined leaf-necrosis (especially of the older leaves) without lesions on the petioles or stem.

Each of these potato viruses has been mixed with *tobacco virus No.* 1 and inoculated into tomato. Streak resulted in every case, though it was interesting to note that the intensity of the streak symptoms varied, the symptoms being most intense when the most virulent potato virus was used.

DISCUSSION AND CONCLUSIONS.

The results reported above show that the tomato mosaic, common tobacco mosaic, yellow mosaic of tomato, single-virus streak and mixed-virus streak diseases which occur in both England and Canada are caused by the same viruses or virus mixtures.

Of the fourteen samples of Canadian streak material examined two were found to be of the mixed-virus type, and from one sample *tobacco virus No.* 1 only was isolated. The presumption is that in this latter material, the potato virus fraction if present had been lost during drying and transportation. This would suggest that a mixed-virus streak of the tobacco-potato mosaic virus type is more common in Canada than it is in England where it is rarely found. Although the results obtained agree in this particular with the view expressed in Canadian literature that a mixed-virus streak of this type occurs under commercial conditions in Canada, they tend, in the main, to indicate that the usual cause of streak is the single virus which is commonly found under English conditions. This virus appears to be as often found in Canadian material as in English. Of the two samples of mixed-virus streak in which both components of the mixture were isolated the mixture was not the classical one of *tobacco virus No.* 1 and a potato mosaic virus, but *tomato streak virus No.* 1 and a potato mosaic virus of the *X* type. As has been shown, the former virus of itself causes streak in many cases but may induce a disease of the mosaic type, without necrosis. Combined with *potato virus X*, however, it produces, invariably, definite streak lesions.

Ring mosaic virus and *tobacco virus No.* 9 cause distinct diseases which have not so far been recorded as occurring in England.

SUMMARY.

It has been found from a comparative study of English and Canadian tomato virus diseases that the commoner viruses which affect the tomato occur in both countries.

Tomato streak caused by the same single virus occurs in both countries, but streak due to a mixed-virus infection appears to be more frequent in Canada than in England.

The following diseases with their causal viruses are described in this paper. Tomato mosaic (*tobacco virus No.* 1), yellow mosaic of tomato (*tobacco virus No.* 6), single-virus streak (*tomato streak virus No.* 1), mixed-virus streak (a tobacco virus + a potato virus) and spotted wilt (*spotted wilt virus*). The presence of the last-named disease in Canada was not established.

Ring mosaic virus and *tobacco virus No.* 9, which have not been recorded in England, are also described.

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EXPLANATION OF PLATES XXVI AND XXVII.

PLATE XXVI.

Fig. 1. Healthy tomato leaf (var. E.S. 1).

Fig. 2. Tomato leaf (var. E.S. 1) showing symptoms of tomato mosaic.

(The leaves illustrated in Figs. 1 and 2 are reproduced on the same scale and were corresponding leaves from plants of the same age grown under the same conditions.)

Fig. 3. Tomato leaf showing symptoms of yellow mosaic of tomato.

Fig. 4. Tomato leaf showing symptoms of ring mosaic streak.

PLATE XXVII.

Fig. 5. Tomato plant showing symptoms of single-virus streak.

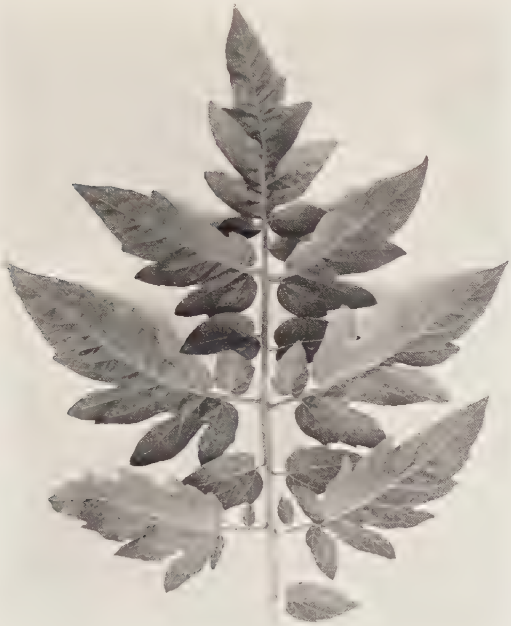
Fig. 6. Green tomato fruit showing markings caused by *tomato streak virus* No. 1 (single-virus streak).

Fig. 7. Tomato leaves showing bronzing symptom of spotted wilt.

Fig. 8. Green tomato fruit showing symptoms of spotted wilt.

Fig. 9. Ripe tomato fruit showing yellow markings caused by *tobacco virus* No. 6 (yellow mosaic of tomato).

(*Received July 4th*, 1934.)



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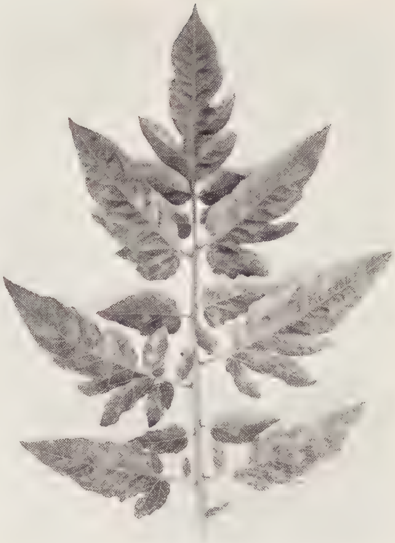

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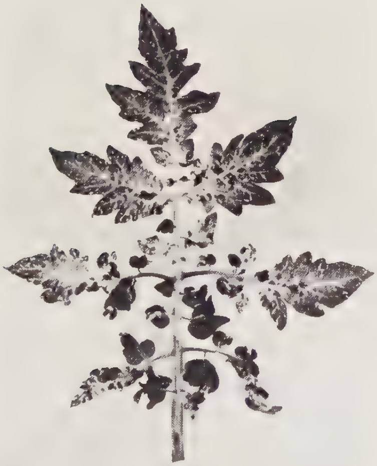

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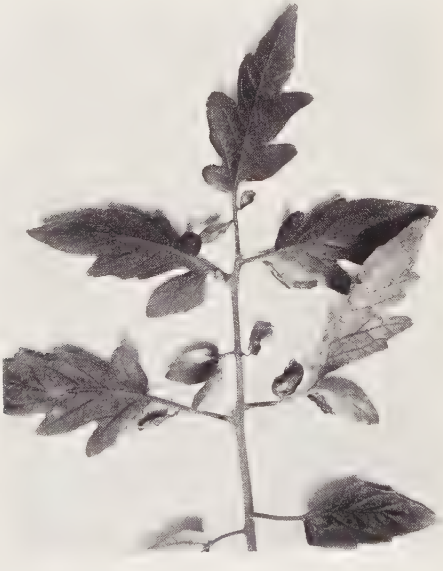
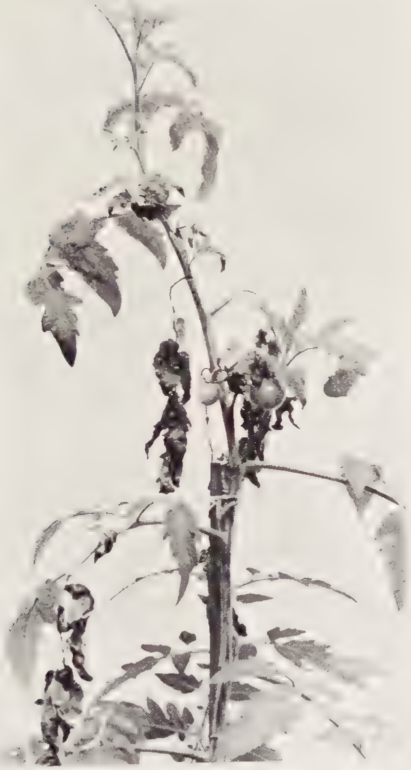


Fig. 4.

AINSWORTH, BERKELEY AND CALDWELL.—A COMPARISON OF ENGLISH AND CANADIAN TOMATO VIRUS DISEASES (pp. 566–580).



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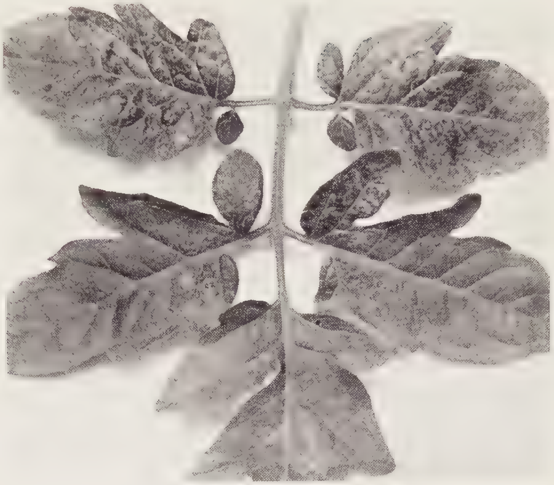

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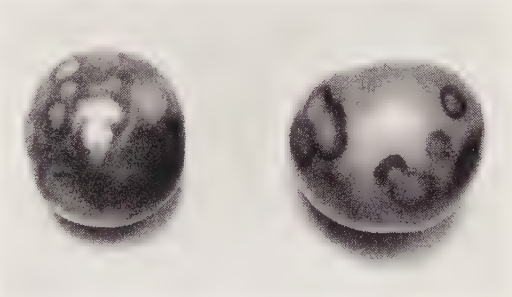

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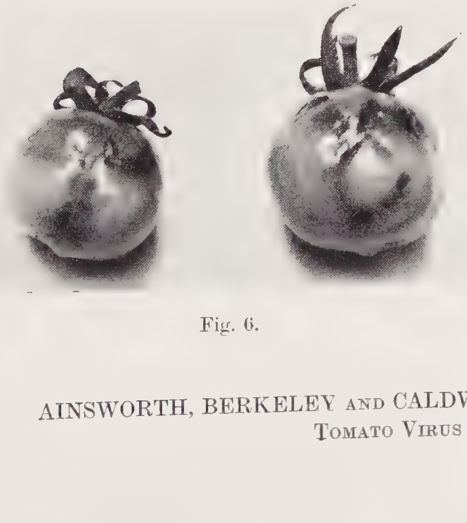

Fig. 6.

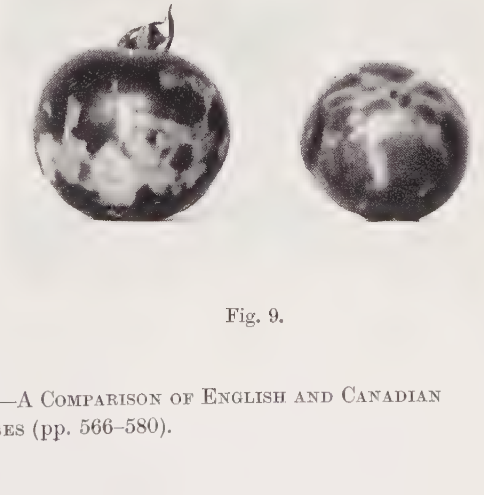

Fig. 9.

AINSWORTH, BERKELEY AND CALDWELL.—A COMPARISON OF ENGLISH AND CANADIAN TOMATO VIRUS DISEASES (pp. 566–580).

A COMPARISON OF CERTAIN ENGLISH AND CANADIAN POTATO VIRUSES FOUND INFECTING TOMATOES

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(With Plate XXVIII.)

Introduction.

During the examination of virus-diseased tomato material from Canada (a detailed account of which is given by Ainsworth, Berkeley and Caldwell (1)) several isolations of potato viruses were made. In order to establish the identity of these viruses they were compared with standard English material, and the results of this study are reported in the present paper.

The "latent" and the "virulent latent" potato viruses.

Burnett and Jones (3) in their investigation of the effects of certain potato viruses on tomato plants distinguished two types of the latent potato virus as the "latent" and the "virulent latent." Both these types, which produce distinct symptom pictures on tomato, have been studied.

The "latent" virus causes a mild mottle on tomato without leaf distortion or stunting. Pale green or yellow areas develop between the veins, and there is often more or less distinct vein-banding (Plate XXVIII, fig. 2). There is usually no necrosis, but a few minute necrotic lesions sometimes develop on the lower leaves. The symptoms on tomato can be almost imperceptible, and a plant that has been inoculated six to eight weeks may "carry" this virus perfectly. On tobacco a very mild general mottle without malformation or ring patterns is produced.

The "virulent latent" on tomato usually gives rise to spot-like necrotic local lesions followed by a mottle of similar type to that produced by the "latent" virus but more pronounced, and in addition small necrotic flecks, which may be ring-like, appear on the leaves (Plate XXVIII, fig. 4). The necrotic spots first appear on the older leaves and then spread acropetally and may be so numerous that leaves are killed.

Lesions on stem and petioles are not usual (cf. Streak). A plant some weeks after inoculation may successfully "carry" this virus. On tobacco ring and line patterns are produced.

From the literature it appears that the *"virulent latent" virus* of Burnett and Jones (3) is the same as the *healthy potato virus* of E. M. Johnson (5), the *tobacco ring spot virus* of J. Johnson (7) (which is quite distinct from the ring spot virus of Henderson and Wingard (4)) and *potato virus X* of K. M. Smith (11).

The "latent" virus is possibly the same as J. Johnson's mottle virus of tobacco (6), though increase in virulence has not been obtained by transfers through tobacco, while Valleau and Johnson (13) consider it to differ from the "virulent latent" only in virulence, and the evidence presented in this paper supports the latter view.

MATERIAL.

From the various samples of Canadian tomato material examined three isolations of potato viruses were made. Two (from Niagara, Ontario and British Columbia) isolated at Cheshunt, approximated to the "latent" virus, while one (from Niagara, Ontario) isolated by G. H. Berkeley at the East Malling Research Station, was nearer the "virulent latent." These viruses, together with a virus isolated from mosaic-diseased Irish Cobbler potato material from New Brunswick, were compared with:

(1) Authentic *potato virus X*, kindly supplied by Dr K. M. Smith.

(2) A strain of *potato virus X* used at Cheshunt to produce mixed-virus streak. This virus, isolated from garden potatoes in 1931 and kept under observation since, during which time it has been filtered and passed through *Datura Stramonium*, has agreed closely with the published description of the *X* virus.

(3) The potato virus fraction of a mixed-virus tomato streak from Ireland examined at Cheshunt in 1933.

COMPARATIVE STUDY OF THE DIFFERENT VIRUSES.

Properties. All these viruses were filterable unchanged through L1 and L3 but not usually L5 or L7 Pasteur-Chamberland filters, and were inactivated by heating to 70° C. for 10 min. No critical tests were made of their resistance to ageing, but they are definitely less resistant than the tobacco mosaic group of viruses. K. M. Smith (12), p. 310 states that *potato virus X* was active when tested after storage *in vitro* for six weeks at room temperature in a sterile L3 filtrate, and the Cheshunt strain of this virus survives three months, but less than six under similar conditions. Burnett (2) found from a large series of tests that the longevity of the latent virus in dry plant tissue varied with the plant, the virus having survived up to 286 days in dried tobacco leaves, 263 days in potato, but not more than 50 days in tomato. Burnett also found that when the latent virus was dried in combination with tobacco mosaic virus it remained infective for a longer time than when dried alone; up to 618 days in tobacco, 352 days in potato and 1251 days in tomato tissue. From one sample of dried Canadian tomato streak material potato virus was isolated on December 19th, again from the same material on January 16th, but when tested for the third time on March 26th only the tomato virus fraction of the streak was detected. That is, the material having been collected about a month before the first test, the potato virus fraction survived less than four months' drying.

Symptoms. A comparison of the symptom pictures produced by these viruses on tomato (var. E.S. 1), tobacco (var. White Burley), *N. glutinosa* and *Datura Stramonium* was made in two successive series of parallel inoculations. These series showed that the intensity of the symptoms varied on all the host plants from the somewhat virulent symptoms, typical of the *X* virus, to the mild symptoms characteristic of the "latent" virus as shown in Table I. Plate XXVIII, figs. 2 and 4 show the range of symptoms obtained on tomato, which are described in more detail above. The symptoms produced by both the mild and the virulent types varied slightly with the season, being more severe in the early spring than later in the year when the temperature of the experimental greenhouse was at times rather high. Under all conditions the difference between the two extreme types was very marked.

Table I.

Comparison of potato viruses.

Virus	Tomato	Tobacco	*N. glutinosa*	*Datura Stramonium*
1. *X* virus (K.M.S.)	Mild mottle, conspicuous leaf necrosis	Definite ring patterns	Mottle, necrosis	Vein-banding, necrosis
2. Potato virus (Ireland)	”	”	”	”
3. *X* virus (Cheshunt)	”	”	”	”
4. Irish Cobbler virus	Mild mottle, leaf necrosis	Few rings	Mottle, very slight necrosis	Vein-banding, very slight necrosis
5. Potato virus (Niagara, Malling)	Mild mottle with mild leaf necrosis	Rather inconspicuous ring pattern	”	”
6. Potato virus (Niagara, Cheshunt)	Mild to almost imperceptible mottle, no necrosis	No ring pattern, mild mottle	Mottle, inclined to vein banding, no necrosis	Vein-banding, no necrosis
7. Potato virus (British Columbia)	”	”	”	”

Each of the potato viruses was mixed with *tobacco virus* 1 and tomato plants inoculated. Typical streak resulted in every case, but the intensity of the symptoms varied, being most severe when the most virulent potato virus was used and least severe with the mild "latent" virus. Plate XXVIII, fig. 1*a* and *b*, shows the variation in the intensity of the streak.

As a result of this comparison all the viruses studied were considered to be of the *potato virus X* type, and additional support for this view was obtained by the protective inoculation experiments dealt with in the next section. The virulent and mild types will be referred to as the *virulent X virus* and the *latent X virus* respectively.

PROTECTIVE INOCULATION OF TOMATO PLANTS AGAINST VIRULENT STRAINS OF THE *X* VIRUS.

It was found that a plant inoculated with the *latent X virus* could not afterwards be infected with the *virulent X virus*, and several experiments of the type described have been performed.

A batch of twenty-eight young tomato plants of the same age was taken and fourteen were inoculated with the *latent X virus*, three with distilled water as controls and the remaining eleven kept untreated. Ten days later when all fourteen plants inoculated with the *latent X virus* were showing a mild mottle, eight were inoculated with the *virulent X virus* and three with the *latent X virus* for the second time. Eight plants of those untreated at the beginning of the experiment were inoculated with *virulent X virus* and three with *latent X virus*. After a further ten days the plants inoculated with the *virulent X virus* only, showed necrotic local lesions on the leaves inoculated and a systemic mottle with leaf necrosis, while those plants which had been previously inoculated with the *latent X virus* showed no local lesions on the leaflets inoculated with the *virulent X virus* and a mild mottle of no greater intensity than that of plants inoculated once (or twice) with the *latent X virus*. To test whether the *virulent X virus* had multiplied to any appreciable extent within the protectively inoculated plants half of all the different series of plants were inoculated with tomato mosaic virus (*tobacco virus* 1). Every plant so inoculated developed streak symptoms, but in all plants inoculated with the *latent X virus* or with the *latent X virus* followed by the *virulent X virus* the streak symptoms were very mild (see Plate XXVIII, fig. 1*b*), while those inoculated with the *virulent X virus* showed severe streak symptoms as typically produced by that virus in combination with *tobacco virus* 1 (see Plate XXVIII, fig. 1*a*). It is concluded that the *virulent X virus* does not multiply in plants previously inoculated with the *latent X virus*.

A few other experiments have been carried out, from which it was found that a plant inoculated with the *latent X virus* may be inoculated on the *same leaflets* with the *virulent X virus* before showing any symptoms due to the former virus, and yet show only mild symptoms. If a plant is inoculated with a mixture of the virulent and latent viruses at the same time the symptoms exhibited are those of the virulent virus, and further inoculations from such a plant have failed to detect the presence of the latent virus. If a seedling tomato plant with four to five leaves is inoculated at the same time with the virulent and latent viruses separately, on the terminal leaflets of the first and second foliage leaves respectively necrotic local lesions form on the leaflet inoculated with the virulent virus, but not on that inoculated with the latent. The systemic symptoms are those characteristic of the virulent virus which is the only virus to be detected in the upper parts of the plant, though the latent virus can still be recovered in a pure state from the original leaflet inoculated in spite of the fact that one is, in the ordinary way, able to detect virus in all parts of a seedling plant inoculated by the *virulent X virus* on one leaflet. The *virulent X virus* appears to be unable to enter a cell infected by the *latent X virus*, or at least to multiply sufficiently in such a cell to be detected by the tests employed.

Salaman (10) has already recorded a very similar case to the one reported above of protective inoculation of tobacco and stramonium plants against more virulent strains of the *X* virus. Salaman obtained his mild strain by punching small segments from the green areas produced by the *X* virus on tobacco leaves and showed that, though this mild strain gave complete protection against the most virulent strains of the *X* virus, it afforded no protection against *potato virus Y* or *tobacco virus* 1. Recently Kunkel (8) has reviewed the literature on immunity acquired by plants against virus diseases and described further cases in which attenuated strains of yellow tomato mosaic (aucuba mosaic) virus protect against more virulent strains of that virus, and attenuated strains of tobacco mosaic against both yellow and ordinary tomato mosaic.

DISCUSSION AND CONCLUSIONS.

From the above results it is concluded that all the different viruses examined are strains of *potato virus X*. The different strains agree in physical properties and will infect the same range of host plants, but differ in the intensity of the symptoms they produce. The fact that mild strains will protect against the virulent is also taken to be evidence in support of their close relationship. The protective action on a plant of one virus against another has not yet been established as a specific reaction, but the instances of protection so far recorded have been between very closely allied viruses.

The different strains studied in the present work have remained constant throughout the time they have been under observation. The two extreme types, typified by the *latent X virus* and the *virulent X virus* as here described, have a very wide distribution, but how these strains arise in nature is problematical. It is possible that the passage of *potato virus X* through a special plant or even certain varieties of potato might cause attenuation, as is the case when virus of curly top of sugar beet is passed through *Chenopodium murale* (Lackey (9)). Or, again, the two types may exist together, as Salaman (10) has experimentally shown to be possible, inoculation by mass transfer masking the mild form, and become separated through the selective action of an insect vector, though at the present time no insect vector of *potato virus X* is known.

It does seem to be advisable that the mild form, the *latent X virus*, should be designated by a special name, Salaman (10) distinguishes it as the *G* type of *X*, for the symptoms it gives rise to on certain plants are dissimilar to those produced by the virulent form which is identical with the type *potato virus X*.

SUMMARY.

Potato viruses isolated from Canadian tomato material have been compared with standard English material.

The different viruses, which corresponded to the mild and virulent forms of the "latent" or "healthy potato" virus, were all considered to be strains of *potato virus X* which differed primarily in virulence.

It was found that immunity against a virulent strain of the virus was conferred on tomato plants by inoculation with a mild strain.

In conclusion I wish to thank Dr G. H. Berkeley to whom I am indebted for all the Canadian material.

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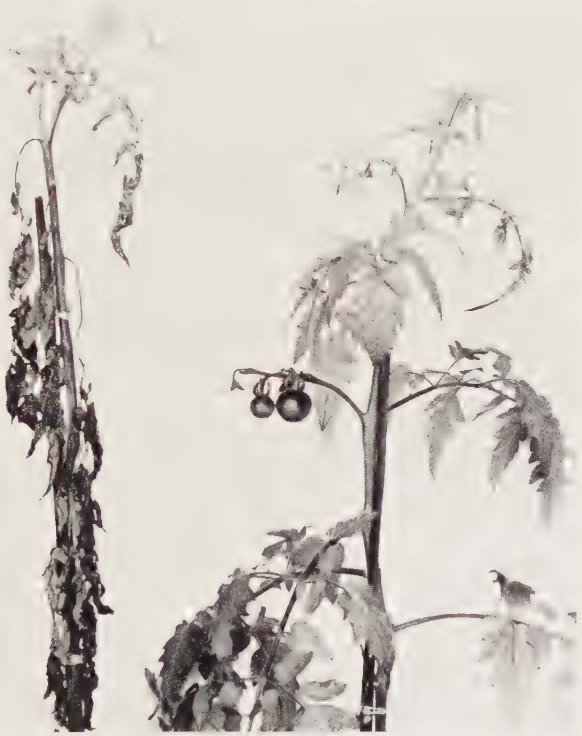


a Fig. 1. *b*

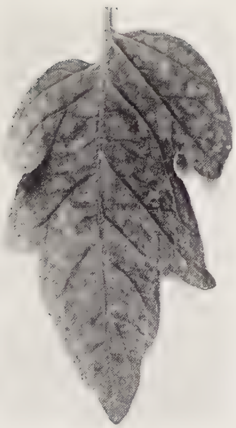
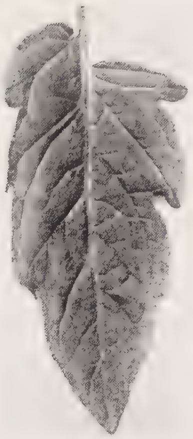


Fig. 2.



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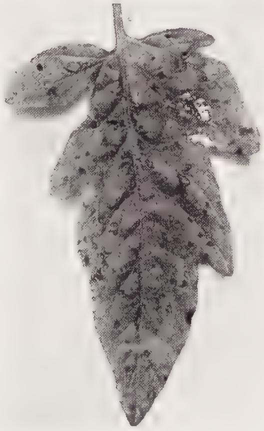

Fig. 4.

AINSWORTH.—A COMPARISON OF CERTAIN ENGLISH AND CANADIAN POTATO VIRUSES FOUND INFECTING TOMATOES (pp. 581–587).

(3) BURNETT, G. and JONES, L. K. (1931). The effect of certain potato and tobacco viruses on tomato plants. *Washington Agric. Sta. Bull.* No. 259.

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EXPLANATION OF PLATE XXVIII.

Fig. 1 *a* and *b*. Tomato plants (var. E.S. 1) of the same age three weeks after inoculation with *tobacco virus* 1, both plants having been previously inoculated on the same day with a virulent strain of *potato virus X*; but *b* had a preliminary inoculation with a mild strain of the potato virus.

Fig. 2. Tomato leaflet showing symptoms produced by a mild strain of *potato virus X*.

Fig. 3. Healthy tomato leaflet.

Fig. 4. Tomato leaflet showing symptoms produced by a virulent strain of *potato virus X*.

The tomato leaflets illustrated in Figs. 2, 3 and 4 were from corresponding leaves of three similar plants inoculated on the same day and grown under the same conditions.

(*Received June 26th,* 1934.)

THE PHOTODYNAMIC ACTION OF METHYLENE BLUE ON THE VIRUS OF A PLANT DISEASE

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Recent work by Perdrau and Todd (1933 *a* and *b*) has demonstrated that certain viruses causing diseases of animals, as well as certain races of bacteriophage, are inactivated *in vitro* by exposure to light in the presence of methylene blue and oxygen. I have found that the virus of a plant disease may be inactivated in a similar way.

The virus of streak disease of maize is not easily handled *in vitro*, since no successful method of mechanical transmission from plant to plant has been devised. A somewhat laborious test for the presence of the virus in a fluid is available however in the method of exposing the fluid (with sugar added to increase palatability) under a suitable membrane to the feeding of the insect vector, *Cicadulina mbila* Naude. On subsequently testing the insects, by cageing singly on maize seedlings, a proportion will be found to have taken up the virus and to have become infective; alternatively, the absence of infective individuals is the best available proof of the absence of active virus from the fluid used. By following this method I have demonstrated the passage of the streak virus through certain grades of filters and its retention by others (1932).

The experiments now described each consisted of three series:

Series A. Sufficient methylene blue (Meister Lucius and Brüning, "medicinale") added to the virus fluid to produce the final concentration required. 5 c.c. irradiated for 3–4½ hours in a Petri dish of 85 mm. diameter in diffused daylight at an open window.

Series B. A similar mixture of virus fluid and methylene blue, but made by the faint green light of a "Panchromatic" dark-room lamp. Kept for a similar period in a Petri dish in total darkness.

Series C. Virus fluid without methylene blue, irradiated as Series A.

At the conclusion of the period of irradiation, each lot of fluid was mixed with an equal quantity of sterile 20 per cent. sucrose by the green light, and exposed for 24 hours in total darkness to the feeding of about twenty adult *C. mbila* of an "active" race (*i.e.* known to be able to transmit the streak virus). The surviving insects were then tested singly on maize seedlings in the light.

The results of five experiments upon these lines appear in the table. While showing some irregularity in the control series, and an unexpectedly low proportion of infections in several, all experiments are consistent in being negative in series A. That this result is not due to the dye having rendered the fluid unpalatable to the insects is shown by the infections in series B. I therefore conclude that the virus has been inactivated in series A by the treatment received.

Tests of Cicadulina mbila *fed on the treated virus fluids.*

Exp. No.	Nature of virus fluid	Final concentration of methylene blue	Series A		Series B		Series C	
			No. tested	No. infective	No. tested	No. infective	No. tested	No. infective
I	Juice, clarified by passage through Kieselguhr bed	1 : 10,000	16	Nil	16	7	16	9
II	,,	1 : 100,000	17	Nil	20	5	16	2
III	,,	1 : 100,000	18	Nil	15	8	18	11
IV	Juice, partly clarified by passage through filter paper	1 : 10,000	19	Nil	14	2	11	2
V	,,	1 : 100,000	7	Nil	3	1	20	16

It is a matter of some fundamental interest that a plant virus is susceptible to the same inactivating influences as animal viruses and the bacteriophage.

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STOREY, H. H. (1932). *Ann. Appl. Biol.* XIX, 1.

(*Received May* 3*rd*, 1934.)

THE LIFE HISTORY AND CULTURAL CHARACTERISTICS OF *TYPHULA GYRANS* (BATSCH) FRIES

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(With Plates XXIX and XXX and 34 Text-figures.)

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I. INTRODUCTION.

THIS investigation of the life history of *Typhula gyrans* was undertaken primarily with a view to determine the degree of pathogenicity of the fungus. The material which formed the basis of the work was obtained from Eastriggs, Dumfriesshire. It consisted of four sclerotia attached to a piece of swede turnip petiole. This was found in a field crop in early January. Subsequent work has shown that the fungus is in all probability only a saprophyte in this country. As this became increasingly clear more attention was paid to the cultural characteristics and to special points, *e.g.* the development of the sclerotium, and the constancy of the characters generally used in determining the genera of the Clavariaceae.

Typhula gyrans (Batsch) Fries is one of a genus placed in the Clavariaceae by most authors. The systematic position of the family differs in many classifications. It is placed in the *Agaricales* by Clements and Shear and Clements (13).

The fungus was first recorded by Bergius (4), who in 1765 observed the sclerotial stage and named it *Lycoperdon Brassicae.* Many other botanists writing towards the close of the eighteenth century have mentioned the sclerotial stage, using a wide variety of names. Batsch (3) recorded the sclerotial and sporophore stages together as *Clavaria gyrans* in 1786. Under this species he included the *Sphaeria Brassicae* of Dickson (15) which comprised ten forms including *Lycoperdon Brassicae* of Bergius. As late as 1860, however, Berkley (5) still recorded the sclerotial stage alone under the name of *Sclerotium semen.* Fries (16) was responsible for the formation of the genus *Typhula.* In 1821 (17) he described eight species including *T. gyrans.* He placed *Clavaria gyrans* Batsch, and *Cl. granulata* Willdenow (40) in this new species. In establishing the genus Fries used fungi from several genera—*Himantia*, *Chordostylum*, and *Clavaria*—and in grouping them together admitted that he was more struck by the general resemblance of their habit than by any particular characters. This same difficulty in defining the genus has been experienced by later writers. Saccardo (34*a*) and Rabenhorst (29*a*) were both struck by the easy gradation of forms from *Clavaria* through *Typhula* to *Pistillaria.* Saccardo (34*a*) gives as the distinguishing character between these two last that *Typhula* has two- or four-spored basidia, while *Pistillaria* has two-spored only. Massee (25) and others are inclined to make use of the separation of the sporophore into a definite head and a stalk—"separated from *Clavaria* and *Pistillaria* by having the stem distinct from the hymenium." Absence of branching in the sporophore is another point on which stress has been laid. Under cultural conditions *Typhula gyrans* has been found to produce sporophores which show no separation into stalk and hymenium, and others which branch freely. This would seem to indicate that the generic differences in the Clavariaceae are not very fundamental in nature.

Saccardo (35) lists sixty-four species of *Typhula.* Spore size has been determined for relatively few of these, and the criteria used in establishing the species are either those of host or habit. The range of hosts on which the species *gyrans* alone has been recorded (pp. 591–2), and the wide variety of forms in which it has appeared in culture, show the slight importance which can be attached to either of these in diagnosis. Spore size may turn out to be the only stable character in this group.

Typhula gyrans has been recorded in Germany (7*b*), Denmark (24), Norway (22), France (10), England (12), Scotland (38), and the United States of America (34*b*). Rostrup (32, 33) only has recorded it as a parasite, on cruciferous crops in Denmark. Lind (24) quotes Mortensen (26) as recording the fungus on turnips in the pits. Withering (41) also records it on turnip roots. Dickson (15) and Withering (41) observed the fungus on the roots of parsnips. Jørstad (22) describes it on cabbage, carrot, and celery.

In all other cases there seems to have been no question of the fungus doing any damage. Most writers merely state that it is to be found among dead leaves. Bolton (6), Dickson (15), Rebentisch (30), de Candolle (11), Berkley (5), and Lind (24), however, record the fungus on dead leaves of Brassicae or more specifically of cabbage. Greville (20) and others describe the sclerotia on dead potato stalks; while still other hosts are listed by de Schweinitz (36) "in caulibus Liliorum," Massee (25), and Lind (24) dead stems of grasses, Lind (24) the pods of *Pisum sativum*, and on *Scorzonera hispanica*. It must be emphasised that in some of these cases at least sclerotia only were observed, and the possibility of error must be kept in mind.

II. INFECTION EXPERIMENTS.

In view of Rostrup's record the pathogenicity of the fungus was investigated in a series of experiments. Attempts were made to infect turnips in all stages of development with all stages of the fungus. The results of these experiments, which are given below, were entirely negative.

(1) In April 1932 swede turnips of the previous year's crop were inoculated with mycelium. Pieces were cut out of the roots and petioles, and the mycelium was inserted. The turnips remained apparently healthy. They were allowed to flower, and the seed was collected and sown out. It germinated in January 1933 giving perfectly healthy seedlings.

(2) In May 1932 attempts were made to infect seedling turnips.

A. Large pieces of mycelium were placed on the surface of the soil in pots containing turnip seedlings at the cotyledon or first-leaf stage. The pots were kept fairly moist and the mycelium spread considerably. The seedlings, however, were never visibly affected, and had formed perfectly healthy plants when last examined in late December 1932.

B. At the same time pots of seeds were set up which contained ten swede seeds each, with, in addition, twenty mature sclerotia. These latter germinated readily producing about one dozen sporophores when the seedlings were at the first-leaf stage, *i.e.* about a fortnight after sowing (Plate XXX, fig. 6). In spite of this exposure to a massed attack the plants produced all remained healthy.

(3) The first experiment was repeated using a variety of white turnip. In some cases sclerotia were inserted instead of pieces of mycelium. No infection was produced.

(4) In April 1933 another attempt was made to bring about infection, this time under more natural conditions. Two drills, each three yards long, were sown in open ground, with a mixture of swede seed and sclerotia. This experiment was duplicated at the Royal Botanic Gardens, Edinburgh, and at Peebles. One hundred sclerotia were included in each sowing. This was a particularly dry, warm period in Edinburgh, and the seeds germinated rapidly. No sporophores were observed at this time, however, or subsequently during weekly inspections. In the case of the Peebles experiment the soil was damper and cooler. The seedlings took a fortnight to reach the cotyledon stage, and by that time twelve spore-bearing sporophores had appeared. From May 9th till October 5th there were always sporophores observed on weekly examination, except for one period of one week at the beginning of July. Apparently the damper climate suited the fungus better. So far, however (May 1934), there have been no signs of sclerotia, sporophores, or mycelium on the living, or on the now abundant dead turnip tissues.

Nearby rows of cabbage and brussels sprout remained unaffected.

III. METHODS.

The preparation of the agars used for general culture work was based on the formulae given by Gwynne Vaughan and Barnes (21 *c*). The fixatives and stains employed were used as recommended by these authors.

Fixation and embedding. Undisturbed mycelium for examination was fixed in 50 per cent. spirit. In the case of sclerotia and sporophores acetic alcohol, strong and weak chromacetic mixtures, Fleming's fluid (strong), and Fleming's fluid ($\frac{1}{2}$ strong) were used. All except the last-mentioned were found to shrink the material rather drastically. This was more obvious in the case of the sporophores. Where embedding was to follow fixation, material was transferred, first of all, by the usual steps to absolute alcohol, xylol, and wax. Latterly, however, it was taken from absolute alcohol to tubes which contained alcohol resting on a layer of cedar-wood oil. After the material had sunk into the oily layer it was transferred to wax. It was frequently necessary to change the wax twice before the oil was completely removed. By this method it was found that material, particularly of sclerotia, was rendered very much less brittle.

A combination of the cedar-wood oil method with the use of wax of melting point 50° C. gave the most satisfactory results.

Staining. Mycelium was stained with erythrosin glycerine, an eosin mounting medium, safranin and light green, or iron alum haematoxylin, and mounted in glycerine jelly.

Embedded material was cut at 2μ or 5μ, usually the latter, and stained with iron alum haematoxylin, or Breinl's triple stain; cleared in cedar-wood oil, and mounted in canada balsam. Preparations of spores were obtained by smearing the fresh sporophores on slides treated with egg albumin, and staining as above.

Examination of fresh material of the fungus in all stages was carried out in mounting medium.

IV. MYCELIUM.

The type of mycelium produced by *Typhula gyrans* in culture varies considerably, depending on humidity, temperature, and the medium employed. Under the most favourable conditions for growth an entirely white spreading mycelium is produced. The hyphal filaments appear as fine threads radiating out from the point of inoculation (Plate XXIX, figs. 2 and 8).

To determine which medium gave the most satisfactory type of growth for general culture the following were experimented with under the same uniform conditions:

(1) Agars: malt, oat, turnip, prune, potato, soil extract.

(2) Sterilised and untreated material from petioles, leaves, and roots of swede and white turnips.

(3) Sterilised soil.

Malt agar proved to be by far the most satisfactory medium for obtaining a clean, rapid growth, and has accordingly been generally employed. A strong clean growth was always readily obtained. Sclerotia and sporophores formed freely. The hyphae were very little raised above the surface of the agar in the first-formed mycelium. The hyphae show as radiating lines spreading out from the centre of inoculation. The mycelium on the other agars varies in abundance, but is otherwise very similar. The growth produced on sterilised soil was sparse. The growth produced on sterilised turnip material was remarkable for the abnormal structures which made their appearance. These are discussed under sporophores and sclerotia. Growth produced on the unsterilised material exhibited no special feature of interest.

Soil survival. The following additional facts were ascertained relative to soil survival under laboratory conditions:

(1) Where sufficient water was provided, masses of sclerotia sown in large dishes germinated, producing both sporophores and mycelium. After seven months these sclerotia were still capable of producing fresh mycelium and sporophores.

(2) Sclerotia were frequently germinated in soil to produce sporophores for fixing and embedding. Six weeks after planting, in one case, mycelium growing from these sporophores over the soil surface produced small white sclerotia which matured in the usual way.

Judging from this evidence it seems probable that, under the more favourable conditions occurring naturally, the fungus may survive for long periods in soil which contains a reasonable amount of organic matter.

The mycelium on any of the agars shows a faint but none the less definite zonation. This takes the form of one to several rings with the point of inoculation as their centre. This phenomenon is not controlled by a light factor, or by temperature. Large numbers of agar plates were exposed to different fixed or varying temperatures; while still others were exposed to continuous light or continuous darkness, or alternating day and night. Zonation was produced in every case. Where cultures are made in tubes on agar slopes the zones appear as bars across the medium. Zonation is widespread among the fungi, and negative results, similar to those mentioned above, have been obtained by Stevens and Hall(37). Gwynne Vaughan and Barnes(21*a*) suggest that it is a chaemotropic reaction due to the reduction in growth produced by the deposit of katabolic substances in front of the advancing hyphae, followed by a renewal of active growth when a few hyphae break through the "staled" region and form a "new ring of richly branched mycelium."

As the mycelium grows older, usually from about three weeks onward, it becomes a little more raised above the surface of the medium and is woollier in appearance (Plate XXIX, fig. 2). There is no microscopical difference in the size or construction of these later-formed hyphae.

In some of the earlier cultures a dense felt of velvety brown mycelium made its appearance. This was very slow-growing (Plate XXIX, fig. 7). The mycelium was uniform in appearance, and it was not possible to distinguish individual threads without a microscope. Subcultures frequently gave rise to normal white growth. These early cultures were made either from entire sclerotia or from parts of them. It therefore appeared possible that the colour of the hyphae might have been communicated to them in some way from the dark rind of the resting bodies. Later observations showed that ordinary white mycelium when subcultured sometimes gave rise to cultures composed entirely, or in part, of brown hyphae. This led to the checking up of the variable conditions, namely temperature and humidity. It was found that the brown mycelium made its appearance on new media only if the cultures were incubated at 20° C. or over. It appeared when old medium, which was very dry, was used, even at lower temperatures. These facts suggested that the brown mycelium was produced in response to dry conditions of growth. This was shown to be the case by growing the fungus at 25° C. in a very moist atmosphere. The normal white mycelium only was produced.

The optimum temperature for growth of the fungus in culture was also investigated. The maximum growth of mycelium was obtained at 15–17° C. The best temperature for sporophore formation was found to be slightly higher, namely 17–20° C., that for sclerotia slightly lower at 13–15° C.

During December 1933 and early January 1934 agar plates inoculated with mycelium were exposed to outside weather conditions. Though no very severe frost was experienced the average minimum temperature over the period was 0·5° C., and several degrees of frost were usually registered at night. In all cases the fungus not only remained alive, but grew a considerable amount, producing some sclerotia.

These temperature experiments show that the range within which the fungus may be expected to survive in the form of mycelium is from 25° C. to below 0° C. No doubt survival in the resistant sclerotial form is possible over a considerably greater range.

The most striking feature of the mycelium, when it is examined microscopically, is the presence on the hyphae of numerous clamp connections. These are formed in the following manner. A hooked or beaked projection appears on any part of the hypha, and, curving away from the growing point of the thread, unites with the same filament at a point further removed from the apex. The clamp connection is always associated with a cross-septum in the hypha forming it. This transverse wall is usually present before formation of the clamp begins (Text-fig. 2). In some cases it is not completed until after the protuberance has appeared (Text-fig. 1). Rarely it is only produced after the clamp connection has fused with the parent hypha (Text-fig. 4). No matter when the cross-wall is formed the clamp invariably has its origin above it, and rejoins the hypha below it. Brefeld observed this phenomenon in the case of *Coprinus*; and caused de Bary (2*a*) to speculate whether or not this type of formation would be found to be the invariable rule.

The wall of the tip of the clamp disappears, making a temporary passage between the two hyphal cells (Text-fig. 3). Very shortly afterwards a septum appears in the clamp itself, usually in its basal portion, and in this way the passage is apparently closed (Text-fig. 5).

The cytoplasm of the vegetative hyphae is granular in character, containing particles of varying size scattered throughout. There is a tendency, in rapidly growing mycelia, towards concentration of the cytoplasm in the tips of the hyphae.

The mycelium branches freely and is many-celled. The length of the cells varies considerably. Many of the septa between hyphal cells are not associated with clamp connections (Text-fig. 15). Branches frequently, but not always, arise from cells which show clamp connections. Where the latter do occur connected with branching, the sequence of events leading to formation is as follows: A clamp connection forms in the way already described. Afterwards a bulge appears on the hypha (Text-fig. 8), or on the clamp (Text-fig. 9), or on both at the same time (Text-fig. 10). In any case the swelling is just below the cross-wall. It develops into the new branch. Sometimes another clamp appears on the base of the branch (Text-fig. 11).

When a branch has just begun to form there is sometimes difficulty in distinguishing it from a clamp in process of formation (Text-fig. 12). This led to some confusion at first. Careful observation, however, has always shown that if a protuberance appears on the side of a cross-wall further removed from the apex of a hypha it does not fuse with the hypha producing it.

The function of these clamp connections must still be regarded as not completely clear. Until recently they were assumed to provide the only means of communication between adjacent cells in a hyphal filament, and the idea expressed by many writers was that by their means nuclear migrations took place from cell to cell. The publication of the latest volume of Buller's *Researches* (9) has brought forward the view that they are of secondary importance; the primary means of communication between cells being provided by pores in the septa. These pores allow of the movement of nuclei from cell to cell, and preserve the continuity of the protoplasm. The function of the clamp connections is said to be to provide an additional pore in time of rapid cytoplasm movement, *e.g.* before sporophore formation commences. The writer has frequently observed, in *Typhula gyrans* mycelium, septa with pores. These pores are more or less centrally placed. They have been assumed to be stages in the development of a complete septum whose formation began at the outside walls, progressing inwards till the pore was blocked, and communication between adjacent cells was prevented. Why clamp formation should begin, in some cases, before the cross-septum is complete, or even obvious, remains obscure.

Anastomoses occur between adjacent hyphae of the same mycelium. In some of these cases at least, the entire cytoplasmic content of one cell passes through the connecting tube into the cell of the other hypha (Text-fig. 14).

So far no staining method employed has made it possible to distinguish the vegetative nuclei with certainty. Particular attention has been paid to clamp connections and the tips of rapidly growing hyphae in an effort to clear up this important point.

Hyphae vary in breadth from a minimum of 3μ at the tips to a maximum of 7μ in some of the older parts. The average breadth of apical cells is 4μ, and that of cells on parts of the mycelium where growth has ceased 5μ.

The hyphae composing the brown felt of mycelium vary very little in breadth from those of the white mycelium. The cells are much shorter than those normally produced, and the walls are slightly thicker (Text-fig. 13). The cytoplasm is granular, as in the normal hyphae. The brown colour is due to an oily substance present in the cytoplasm and cell walls. This oiliness is also a feature of the rind of the sclerotium. As growth is invariably very slow—an increase in diameter of an inch in a month being the maximum observed in colonies on agar plates—it seems reasonable to regard these hyphae as forming a modified resting mycelium intermediate between the normal hyphae and the completely resting sclerotial stage. Normal hyphae are rapidly produced on the return of conditions favourable to growth. The brown mycelium would, therefore, appear to be capable of serving the same purpose as the persistent brown mycelium in *Sphaerotheca mors-uvae*, or the resistant cells in *Uncinula necator* and *Microsphaera Alni extensa* described by Appel (1) and Petri (28) respectively.

Hyphae which are massing together in the earliest stages of sclerotium formation, do not differ much in breadth from the ordinary hyphae. They are, however, very much interlaced and knotted (Text-fig. 15).

Cell contents do not appear to be any more abundant in sclerotia-forming hyphae, or in the cells of the brown mycelium, than in normal cells.

No oidia, such as are recorded by Brefeld ((7b), Tafel 8, Fig. 2) for *Typhula variabilis*, have been observed on mycelia of *T. gyrans*.

V. SCLEROTIUM.

The time of appearance of the sclerotia has been variously described as autumn, autumn and winter, winter, winter and spring. It has been found that sclerotia are readily formed all the year round under laboratory conditions.

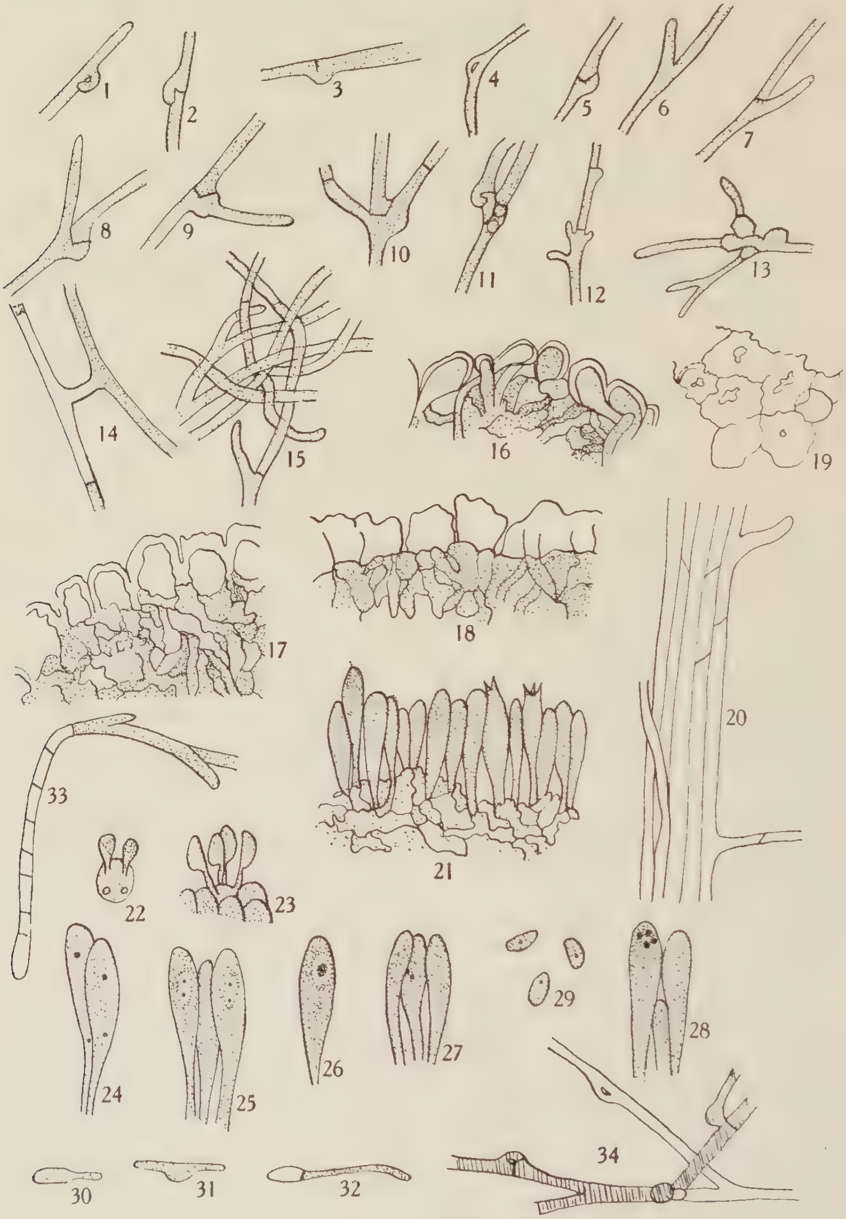
There is considerable variation in size, shape, and even colour of the sclerotia produced in culture. Checks in development are not infrequent, which result in sclerotia ceasing growth at one or other of the immature stages described below. These facts are reflected in the number of names under which the various forms went, particularly before the connection between the sporophore and sclerotial stages was established, and in many of the confusing differences in the descriptions of the earlier writers.

Mature, *i.e.* black sclerotia vary in size roughly from 1 to 3 mm. in diameter (Plate XXIX, fig. 1). Sometimes sclerotia join together to form large masses as recorded by Dickson (15); or even crusts as mentioned by de Bary (2c). The outline of the individuals can be made out more or less distinctly. In some cases they can be separated as complete sclerotia by very gently rubbing the mass between the fingers. In other cases they become firmly fixed together under a common rind with no separating walls. The size of these aggregate masses ranges up to 1 cm. in diameter. Complete drying reduces the size of the sclerotia by about one-third.

Single mature sclerotia are surrounded by a dark rind, which is dry and pitted. They are roughly rounded, and slightly depressed underneath. This depression marks the point of attachment of the sclerotium to the substratum.

The course of development has been traced in culture.

The first sign of sclerotium formation is the appearance of a very minute, dead white point on the surface of the mycelium (Plate XXIX, fig. 2). Brefeld (7a) states that the primordium of a sclerotium is a single branch of a mycelial filament which has rapidly produced a tuft of many branchlets. In this case, however, it appears to be composed of an interlaced mass of branched hyphae produced by many filaments uniting (Text-fig. 15). This denser point next assumes the form of a very small white mass resting on the substratum, to which it is attached by a number of hyphal threads. The "stalk" is too short to be measured. Increase in size takes place accompanied by changes in the colour of the rind. It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Text-figs. 1–34.

becomes in turn pale yellow-white, pale brown, dark brown, and ultimately black. These changes are accompanied by the appearance of a drop of clear-shining liquid on the surface of the sclerotium (Plate XXIX, fig. 2). De Bary (2c) observed this exudation of liquid as a constant

Text-figs. 1–34.

1. Part of a hypha showing a clamp connection forming. No septum is present. × 595.
2. Clamp connection formation. A septum is present in this case. × 595.
3. Clamp connection formation. The tip of the clamp has fused with the hypha. × 595.
4. Clamp connection formation. As in Text-fig. 3, but no septum is present. × 480.
5. Clamp connection formation completed. Septa separate the two cells, and close the clamp. × 595.
6. Branching of a hypha. No cross-wall is present. × 595.
7. Branching of a hypha. A cross-wall is present. × 595.
8. Hypha showing clamp connection with branch beside it. × 595.
9. Hypha showing clamp connection with branch on it. × 595.
10. Hypha showing clamp connection with branches arising both beside, and on it. × 595.
11. Hypha showing clamp connection in surface view. A branch, which bears a clamp connection, is present on one side. × 595.
12. Hypha showing young branches to illustrate their resemblance to clamp connections. × 450.
13. Hypha from brown mycelium. × 450.
14. Anastomoses between adjacent hyphae. The contents of one cell have passed into the other. × 760.
15. Part of a hyphal knot, marking the beginning of sclerotium formation. × 480.
16. Section through outer part of a young white sclerotium. × 445.
17. Section through outer part of a brown sclerotium. × 445.
18. Section through outer part of a mature black sclerotium. × 480.
19. Surface view of the rind of a mature sclerotium. × 480.
20. Longitudinal section of sporophore stalk, showing hairs. × 595.
21. Longitudinal section of sporophore head, showing basidia. × 595.
22. Oblique view of a single basidium to show developing spores. × 760.
23. A basidium showing the four basidiospores and sterigmata. × 760.
24. Developing basidia showing associated nuclei, one in the lower and one in the upper portion. × 760.
25. Developing basidia showing the approach of the associated nuclei. × 760.
26. Fusion nucleus produced by the associated nuclei. × 760.
27. Basidium showing first division of the fusion nucleus. × 760.
28. Basidium showing second division of the fusion nucleus to produce the four spore nuclei. × 760.
29. Mature spores. × 595.
30. Spore germination by a terminal tube. × 650.
31. Spore germination by two terminal tubes. × 650.
32. Germinated spore showing septum cutting off the empty spore. × 650.
33. Young mycelium showing empty spore and seven empty cells. Two branches are already forming. × 595.
34. Fusion between hyphae of two three-day-old monospore cultures. Clamp connections are forming on both hyphae. × 595.

feature of sclerotium formation, and attributed it to the maturation processes going on inside these bodies. Increase in size of the sclerotium ceases when the rind is a uniform brown colour all over. The drop of liquid gradually disappears when the sclerotium has turned black. The surface is left glossy; but this glossiness gradually disappears as the sclerotium becomes older. When dry the sclerotium becomes gritty and carbonaceous in appearance (Plate XXIX, fig. 3). Bolton (6) states that the sclerotia become hollow as decay approaches; but sections through the present material at all stages of development have shown them to be composed of a limiting layer, a white medulla, and a hollow centre. The medulla has approximately the same appearance at all stages of development. The hyphae are much intertwined. Towards the outside they are densely packed, with few or no air spaces. This close packing gives way gradually towards the centre to a looser texture with numerous air spaces. The appearance of the individual hyphae under the microscope varies considerably, and is determined by the direction of the section. In general the medulla suggests a parenchymatous cell tissue. The walls of the hyphae are not conspicuously thickened. The hyphal cells contain large numbers of dark-staining granules (Text-figs. 16, 17 and 18).

The appearance of the rind changes considerably during development. Once the sclerotium has assumed its spherical form a limiting layer is already present. Certain branches of the hyphae composing the hyphal knot grow in such a way that their ends appear free on the outside of the mass. The free ends of these hyphae are swollen in the same manner as the terminal cells which form the basidia on the sporophores, although they are shorter (cf. Text-fig. 21). They form a practically continuous layer (Text-fig. 16). Thickening of this layer takes place early. The outer walls in particular increase in thickness; but the lateral and inner walls are also strengthened. In this way a strong wall without interstices is produced. The "cells" composing it lose their contents and deepen in colour to a very dark brown (Text-fig. 17). The rounded ends of the hyphae are responsible for the rough appearance of the outer surface. The extreme tips of the "cells" are, in many cases, less thickened than the rest of the sloping outer walls. This makes the rind appear to be composed of several layers, and accounts for the roughly concentric rings seen in surface view (Text-fig. 19). The sclerotium increases in size for some time after the outer layer is complete. This is probably sufficiently allowed for by the expansion of the rind produced due to the thickening of the lateral walls. If such were not sufficient it would be an easy matter for new hyphal branches to grow out into any part where the rind cells had been forced apart by the pressure of the medullary hyphae. Sclerotia examined by means of a binocular microscope have always shown a complete surface.

This sclerotium development has been described by Brefeld (7 *a*) and discussed by de Bary (2 *b*) in considerable detail.

In the mature stage the rind is easily cracked, the "cells" splitting away from each other (Text-fig. 18). When the sclerotium germinates it is through one of these cracks that the mass of hyphae pass to the outside. The rind does not take any part in this renewed growth. Some few threads turn downwards into the soil, giving the root-like appearance figured by Bolton (6). The rest of the mass grows upwards to form the young sporophore.

The time taken for the development of the sclerotium, from its first appearance to the mature stage at which the drop of liquid disappears, varies from nine or ten days upwards.

De Bary (2 *d*) states that mature sclerotia pass into a resting stage the duration of which varies with the species, and, in the individual, with internal and external causes. He gives the period of rest for the sclerotia of *Typhula gyrans* as summer. Sclerotia in culture have been germinated immediately on reaching maturity, or at any time up to twelve months afterwards (the longest period tried). Sclerotia sown outside germinated readily during summer, as has been noted under Infection Experiments. Individual sclerotia, however, showed wide variation. Germination has been secured over a period ranging from eight days to five months.

De Bary (2 *e*) also states that the sclerotia of Typhulae, on account of the differentiation of their tissues, do not appear to be able to regenerate rind, or to produce mycelium from rind cells. This is the case for *T. gyrans*. In some cases a drop of liquid was produced on the cut surface of the sclerotium. The only function of the rind, therefore, appears to be protection.

Attention has been drawn already to the zonation of the mycelium. Formation of sclerotia occurs singly or in groups on the hyphae (Plate XXIX, fig. 1). The sclerotia lie on the circumference of one or more rough circles centring at the point of inoculation of the petri dish (Plate XXIX, fig. 1), or in rows on the slopes (Plate XXX, fig. 7). The completeness and number of the circles or rows varies between cultures; but there is obviously a correspondence between the zones on the mycelium and the place of appearance of the sclerotia.

Hyphae frequently grow over the surface of the glass of the culture vessels. Sclerotia occasionally form on the glass.

VI. SPOROPHORES.

The term sporophores is used to include all outgrowths from the mycelium of the fungus, other than sclerotia, whether they actually produce spores or not. The reasons for so doing are given later.

The time of appearance of sporophores in nature is given by most authorities as in autumn or in October, and, in one case, May. Normally sporophores arise from sclerotia. Very frequently in agar cultures structures identical with those produced by sclerotia in the laboratory arise directly from the surface of the mycelium without any kind of sclerotial structure at the base (Plate XXIX, figs. 6 and 7). Swartz (39), Fries (18) and others have noted that sporophores sometimes arise in nature without sclerotia being present.

All sporophores appear as rapidly growing masses of hyphae. They grow vertically upwards, and attain their full size in from one to four days normally. The typical sporophore is a simple unbranched club separable into definite stalk and head portions (Plate XXIX, fig. 5; Plate XXX, fig. 6). The stalk is greyish white and covered by short hairs of varying abundance, which give it a rough appearance to the naked eye. It is clearly marked off from the head by its colour and often by its diameter. The head is completely smooth and dead waxy white in colour. It tapers in some cases to a fairly fine point, in others the apex is blunt. When mature the entire head is covered with spores—the pollen of Batsch (3). The head and stalk are separated as if by a line drawn round the sporophore. The size of the head varies roughly from one-quarter to one-half the total length of the sporophore. Under outside conditions the largest sporophore produced was 2½ cm. from the sclerotium to the apex. The head was 1 cm. long. In pots in the laboratory sporophores reached sizes up to 5 cm., and none were less than 2 cm. No matter from what type of sporophore they are produced spores always vary in size within the same limits.

All sporophores are very susceptible to drying and, when they are exposed to the air of the laboratory, exhibit the curving movements which caused Batsch ((3), f. 164) to give the fungus its specific name. Less than 5 minutes' exposure is sufficient to shrivel them up completely. This process always follows approximately the same steps. The head begins to droop sideways on the stalk. This movement is accompanied by a pronounced lateral corkscrew twisting of the neck portion, until the head is hanging upside down on the end of the wilting stalk. Finally the whole sporophore presents a brownish, shrivelled appearance, and collapses on to the surface of the soil (Plate XXX, figs. 1 and 2). Many mature sporophores show a quite pronounced corkscrew marking on the upper part of the stalk when in a fresh condition. This has been observed and figured by Bolton (6).

The external differentiation into a head and stalk is reflected in the structure observed in microscopic sections. The stalk consists of a number of hyphae, closely packed together, running mainly vertically, but with branches passing among the parallel strands and holding them together (Text-fig. 20). Some of those towards the outside of the bundle have their ends running almost horizontally. These are free from the stalk and form the hairs (Text-fig. 20). The stalk cells are without obvious nuclei, and practically without contents. The length of the cells is variable.

In the head the hymenium completely covers the outside. It is formed by the ends of the hyphae turning outwards, vertical to the surface, and forming a continuous layer of swollen, round-ended cells. These are the young basidia (Text-fig. 21). Apparently every terminal cell is a potential basidium. According to Brefeld (7*a*) the basidium is capable of producing more than one crop of spores. No evidence of this has been forthcoming during the present work. The basidia do not all ripen at the same time, and the life of the average sporophore is so short that a renewal process seems unnecessary.

There do not appear to be any cistidia.

Both the stalk and the head are hollow. This makes a pronounced resemblance between sporophore and sclerotium in section. The fundamental plan of construction seems to be the same in both.

Various nuclear conditions were observed; and the maturation processes in the basidium probably take place by the following steps. When the swollen ends of the hyphae in the head have formed the young basidia they apparently contain two nuclei. One of these is in the lower, and one in the upper part of the structure (Text-figs. 24 and 25). These approach and fuse, usually in the upper part (Text-fig. 26). The single nucleus thus formed divides twice giving four nuclei (Text-figs. 27 and 28). Owing to the small size of the nuclei it has not been possible to distinguish chromosomes; but it will be shown later that a reduction to the haploid number probably takes place at one of these divisions. About this stage of development four sharp points appear at the upper end of the basidium. They form the corners of a rough square. They grow in length, forming stalks, and a swelling is produced at the tip of each (Text-fig. 22). These latter are the young spores. One nucleus passes into each. It has been found impossible to keep spores attached to the basidia during fixation and embedding. For this reason it is very difficult to say when exactly the nuclei pass in. Basidia bearing fully formed sterigmata have not been observed to contain nuclei (Text-fig. 21). It seems probable then that the nuclei pass into the developing spores. The stalks vary in length, being often as long as the spores themselves.

The average spore measurement calculated from fifty spores was $5{\cdot}6 \times 4{\cdot}4\,\mu$. These figures do not correspond with the size given by Patouillard (27)—$5\text{–}6 \times 2\,\mu$. They more nearly approach Massee's figures (25) of $5 \times 6\,\mu$. The present measurements are for fresh spores in mounting medium. Fixed and stained specimens gave measurements much closer to those found by Patouillard. The spores are longer than broad. They are usually rather boat-shaped, tending to be flattened on their inner and rounded on their outer sides (Text-fig. 29). They germinate readily in weak sugar solutions, or on malt agar. A germ tube is produced from either end, or from both ends at once (Text-figs. 30 and 31). The hyphae produced quickly become septate and branch readily. The cytoplasm tends to aggregate in the tips of the hyphae, with the result that the spore (Text-fig. 32), and a number of the cells nearest to it, are left without contents, often within twenty-four hours of germination (Text-fig. 33).

The definite separation of the sporophore into a head and stalk has broken down in culture. Under laboratory conditions long sporophore-like outgrowths are produced from the surface of the mycelium (Plate XXX, fig. 3). These attain lengths up to 12 cm. They are produced in circles or rows in the same way as the sclerotia (Plate XXIX, fig. 6). When young they resemble normal sporophores in colour and shape; but as they grow older they assume diverse forms varying considerably in diameter, and frequently showing a rope-like structure. Some authors have apparently doubted that these structures are true sporophores. Greville (20) describes a similar condition in *Phacorhiza filiformis* (*Typhula Phacorhiza* Fries). He mentions "abortive plants—frequently reaching 5–6 in.—often branched—in diameter scarcely exceeding a human hair. This change appears to be analogous to that in *Agaricus androsaceus*—and which has been described as a *Rhizomorpha*." In a recent paper on *Typhula graminum*, Remsberg and Hungerford (31) have recorded similar structures, regarding them apparently as in no way connected with the sporophore stage of that fungus, and calling them rhizomorphs. Comparison of photographs leaves no doubt that these structures are produced in the same way as those found in *T. gyrans*. Tubes containing these elongated sporophores were placed in a damp, cool greenhouse. After a month they were completely covered by a white waxy bloom. On examination this proved to be composed of masses of basidiospores, produced under the more natural conditions. These corresponded in measurements with spores produced on typical sporophores, and on culturing gave rise to the usual mycelium.

The simple character of the sporophore is not a constant feature either. Short branches have been observed on the sporophores when growing out of doors. These give a stag's-horn-like appearance to the sporophores. In the case of sporophores growing under glass in pots this tendency to branch is more pronounced. The spurs often attain some size, and may even branch again. Side branches only appear when growth has ceased in the original branch. Occasionally a simple sporophore, which has ceased growth and has produced its spores, will start growing again, producing a fresh crop on the new hymenium. Side branches, too, bear the usual large numbers of spores. Branching takes place much more freely on culture media. In extreme cases as many as seven main branches have been counted on a single fructification, together with numerous tertiary spurs. A sporophore typical of this kind is illustrated (Plate XXX, fig. 8). These culture sporophores are hairy all over. When branched they have a stag's-horn or seaweed-like appearance, according to the extent of the branching. The tips are composed, as in young normal sporophores, of a large number of ends of hyphae curved over to form the rounded growing point. In some cases these terminal hyphae have been observed to feather out on coming in contact with the surface of the culture medium, or the glass of the containing vessel. This was also seen sometimes in pot cultures of sporophores. After spore production had ceased and the sporophore had bent over towards the soil, fine threads of mycelium grew out from the surface of the hymenium and stalk. Possibly by this means in nature the fungus can increase its chance of survival.

From these facts it appears that it is a comparatively easy matter for the fungus to pass from the vegetative to the reproductive stage and back again without the intervention of the resting sclerotia.

VII. ABNORMAL STRUCTURES.

Various curious intermediate stages between sporophore and sclerotium have been observed.

Many culture sporophores, which do not arise from definite sclerotia, are early observed to have slight white swellings at their bases. These develop a darker colour as the sporophore gets older. Although they have never been observed to become black they are obviously modified sclerotial forms.

Certain cultures on turnip slices in tubes produced short, thick, white sporophores. These developed round swollen tops, which became brown in colour (Plate XXIX, fig. 3). In another case in which sporophores were developing, black tops appeared at their apices, above slight constrictions (Plate XXIX, fig. 4). Microscopic examination showed both the above abnormalities to have a sclerotium-like structure.

On some turnip agar plates thin sporophores of varying length developed. After a time these began to straggle over the surface of the agar. There some of them developed mature sclerotia at their tips (Plate XXX, fig. 4). In one case the "stalk" was over ¾ in. long.

These facts show that there is no essential difference between the hyphae going to form a sclerotium and those composing a sporophore. Under the uniform conditions of the laboratory the normal sequence of sclerotium—rest—sporophore is upset, and sclerotia, or sporophores directly, arise in the same culture, together with all intermediate stages between the two.

General observation of cultures has shown the sporophores to be negatively geotropic, and positively heliotropic. Their region of growth is apical.

VIII. MONOSPORE CULTURES AND HETEROTHALLISM.

(1) Attempts to obtain monospore cultures were made first of all by rubbing an inoculating needle gently against a sporophore, and then drawing the needle across an agar plate. After twenty-four hours the young colonies were cut out and subcultured. Ten days later the subcultures were examined microscopically. Some contained clamp connections while others did not. Within three weeks all those which had shown clamps produced sporophores or sclerotia. Those without clamps did not. The results are tabulated on the opposite page.

From the cultures without clamp connections four agar plates were inoculated. Two different mycelia were placed on each, a little distance apart. After twenty days the mycelia mixed in one case. Sporophores were present, and the culture contained clamp connections. In the three other cases the mycelia approached but did not mix. No sporophores or clamp connections were present. These three cultures were kept under observation for three months. No change took place.

Date of subculturing 27. ix. 33.

	Date of examination								
	7. x. 33			21. x. 33			20. xi. 33		
Culture No.	Sp.	Sc.	CC.	Sp.	Sc.	CC.	Sp.	Sc.	CC.
A 1	–	–	–	–	–	–	–	–	–
A 2	–	–	×	×	×	×	×	×	×
B 1	–	–	–	–	–	–	–	–	–
B 2	–	–	–	–	–	–	–	–	–
B 3	–	–	–	–	–	–	–	–	–
B 4	–	–	–	–	–	–	–	–	–
C 1	–	–	–	–	–	–	–	–	–
C 2	–	–	×	×	–	×	×	–	×
C 3	–	–	×	×	–	×	×	–	×
C 4	–	–	–	–	–	–	–	–	–

Sp. = sporophores.
Sc. = sclerotia.
CC. = clamp connections.
– = absent, × = present.

The method used to obtain monospore cultures was obviously unsatisfactory; but a number of interesting points stood out. All the cultures did not behave in the same way. Excluding those "monospore" cultures which produced clamps, etc. (these have never appeared when such cultures were made by any recognised method), some pairs of monospore mycelia mixed and fused readily with each other, while in other cases apparently no mixing, and certainly no fusions, took place. The cultures in which the mycelia showed fusions produced clamp connections and, subsequently, sporophores or sclerotia or both. This strongly suggested that the two spores concerned were complementary to each other, while those the mycelia of which would not mix were of the same kind and in some way antagonistic to each other. Such a heterothallic condition with like and unlike monospore mycelia is present among the Rusts and Basidiomycetes generally.

(2) Monospore cultures were then obtained by the dilution method (Gwynne Vaughan and Barnes (21 *b*)). They always produced the pure white mycelium typical of *T. gyrans*; but, although they were examined at intervals over a period of three months, they failed to show clamp connections, sclerotia, or sporophores (Plate XXIX, fig. 8).

(3) Another set of monospore cultures was prepared in the same way at the same time. They were subcultured on eight agar plates in pairs, in the manner already described in Exp. 1. The original cultures were preserved. On two other plates pairs of mycelia were mixed on inoculation. After three weeks three out of the eight plates showed sporophores (Plate XXX, fig. 5). In these the mycelia showed fusions, and clamp connections were present. Sclerotia were subsequently produced. In the other five no fusions had taken place. There were no clamp con-

nections, sclerotia, or sporophores present. These were not produced subsequently (Plate XXX, fig. 9). One of the two plates on which the mycelia had been mixed at inoculation produced sclerotia. It contained clamp connections (Plate XXIX, fig. 2). The other showed mycelium only. There were no clamps present. None of the original cultures contained sclerotia, sporophores, or clamp connections. These latter, along with all cultures which gave negative results, were kept under observation for a period of five months. No change occurred in any of them.

A drawing of a fusion between two monospore colonies grown on filtered agar is included (Text-fig. 34). The colonies were three days old.

These anastomoses were not as a rule accompanied by the passage of the contents of one of the cells into the other. Obviously, however, some nuclear change must take place to affect the resulting mycelia so profoundly.

A similar union of monospore strains has been described by Lehfeld (23) for *T. erythropus*. He was of the opinion that the nucleus from one of the uniting cells passed into the other filament and there divided; one daughter nucleus associated with the nucleus already present to form a binucleate cell, while the other passed along the filament, rapidly dividing and bringing about the binucleate condition in every cell. The appearance of clamp connections was associated with these fusions. Buller (8) at first accepted Lehfeld's interpretation and applied it to those phenomena observed in certain other Basidiomycetes (*Coprinus lagopus*, etc.). This theory does account for the rapid appearance of clamp connections on the mycelia at points distant from the original union. The view expressed by Colson (14) in the case of a heterothallic strain of *Neurospora tetrasperma* seems just as applicable, however. The diploidisation of haploid mycelia in *Neurospora* is attributed to the rapid mixing of the hyphae of the two monospore strains. It is suggested that numerous fusions take place throughout the culture, and are all accompanied by the transference of nuclei.

It is reasonable to suppose that in *Typhula gyrans* the nuclei remain associated in pairs till the basidia have formed, when a pair passes into each. Their probable fusion there, with subsequent division to form four haploid spore nuclei, has been suggested in the part of this paper dealing with the sporophores.

IX. SUMMARY.

1. A description is given of the life history and cultural characteristics of *Typhula gyrans* (Batsch) Fries.

2. The parasitism of the fungus was investigated. Infection experiments yielded entirely negative results.

3. The fungus forms resting sclerotia. The mycelium is either pure white and rapidly growing or, under adverse conditions, dark brown and slow-growing. The sporophore consists typically of a stalk and club-shaped head. The hymenium is borne all over the surface of the head. The sporophore usually arises from a sclerotium.

4. The mycelium contains numerous clamp connections. Their function is doubtful.

5. The development of the sclerotium is traced with special reference to the limiting layer.

6. The sequence of nuclear changes in the basidium is suggested.

7. Various abnormal forms, intermediate in construction between sclerotium and sporophore, are described. It is suggested that a consideration of these, together with the resemblance in structure between normal sporophore and normal sclerotium, proves these latter to be fundamentally the same. The proportion of each formed at any time is governed by external conditions.

8. The fungus is heterothallic. Monospore strains fail to produce clamp connections, sclerotia, or sporophores. When monospore cultures are grown in pairs, in some cases mixing and fusion of the hyphae take place, with subsequent production of clamps, sclerotia, and sporophores; in others no fusions take place.

The writer wishes to acknowledge his indebtedness to Dr M. Wilson for his supervision, stimulating criticism, and continued interest in the work. Among numerous others, thanks are due particularly to Mr E. Wyllie Fenton, of the East of Scotland College of Agriculture, for affording opportunity to carry out much of the work, to Mr Johnston, Librarian at the Royal Botanic Gardens, Edinburgh, and to Miss M. Noble for much interesting discussion.

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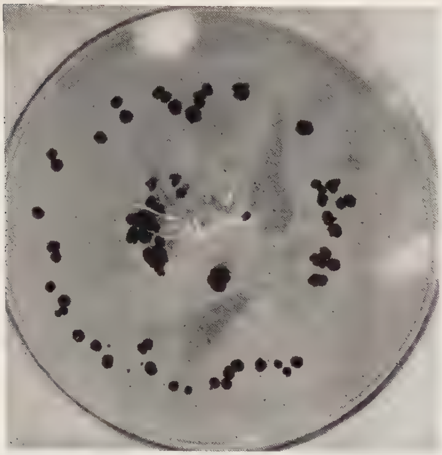

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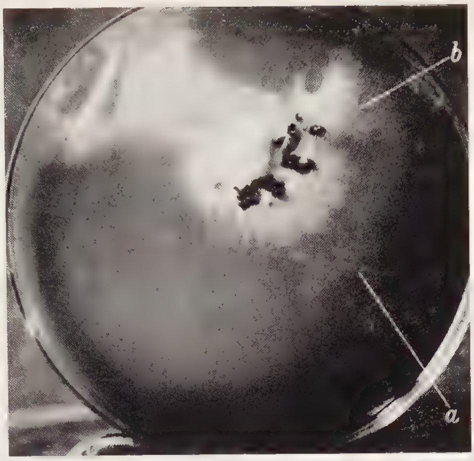

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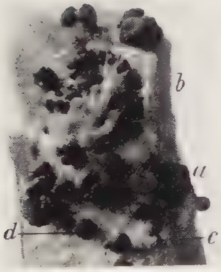

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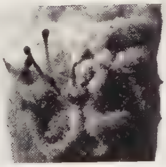

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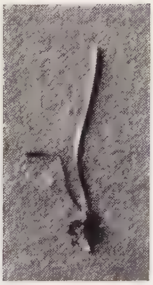

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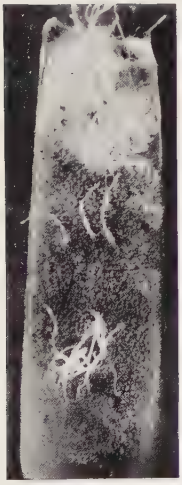

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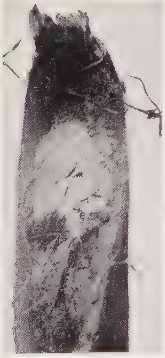

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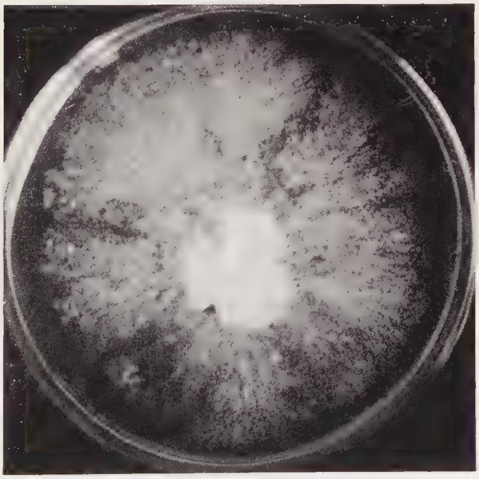
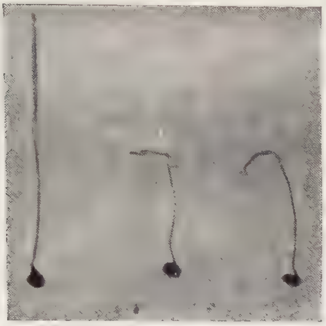


Fig. 8.

MACDONALD.—The Life History and Cultural Characteristics of *Typhula gyrans* (Batsch) Fries (pp. 590–613).



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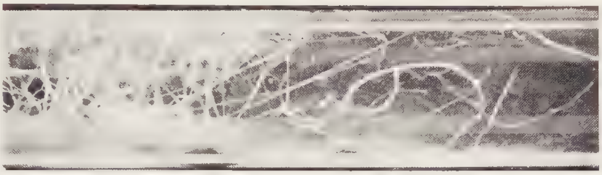

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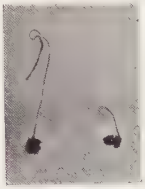

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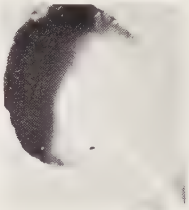

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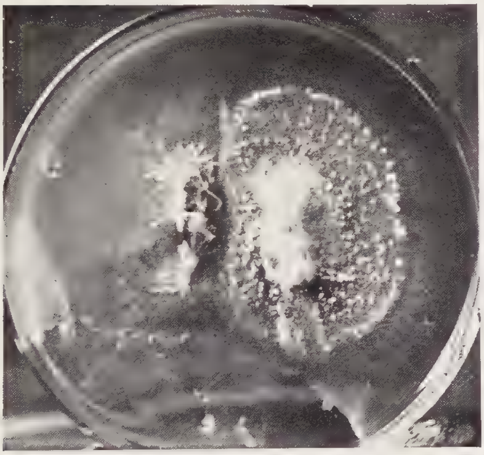

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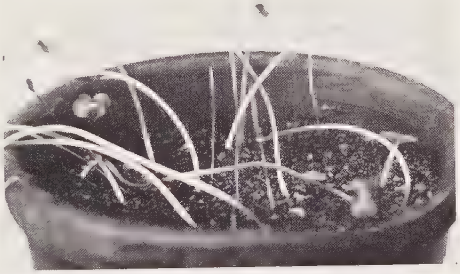

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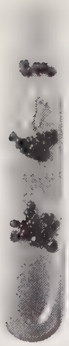

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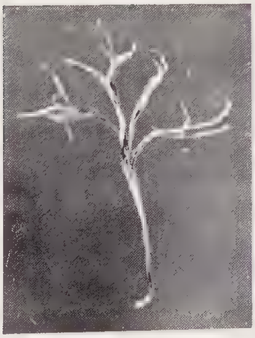

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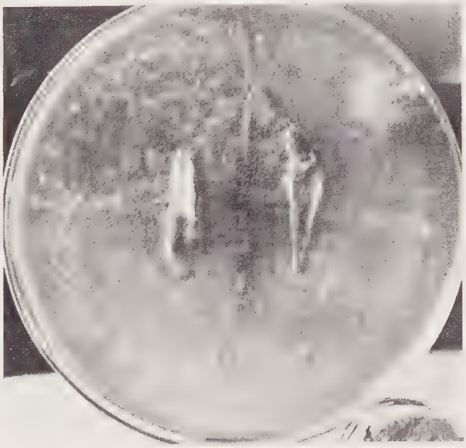

Fig. 9.

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EXPLANATION OF PLATES XXIX AND XXX.

PLATE XXIX.

Fig. 1. Culture showing two rings of sclerotia, which correspond with zones on the mycelium. Straggling sporophores are present in the middle of the plate. × ⅔.

Fig. 2. Two monospore strains mixed at inoculation. (*a*) Primordium of a sclerotium. (*b*) Maturing sclerotium showing exuded drop of liquid. × ⅔.

Fig. 3. Culture on turnip. (*a*) Mature sclerotia showing the wrinkled rind. (*b*) Abnormal structures with brown sclerotium-like heads. (*c*) White sclerotia. (*d*) Mature, dry, carbonaceous sclerotia. × 1½.

Fig. 4. Culture on turnip. Abnormal structures bearing black heads. × 1½.

Fig. 5. Two mature sporophores arising from a sclerotial mass. × 1.

Fig. 6. Three groups of sporophores corresponding with zones on the mycelium. × 1¼.

Fig. 7. Brown mycelium has formed on the dry upper part of the slope. Numerous straggling sporophores are present. × 1.

Fig. 8. Three-months-old monospore culture, showing mycelium only. × ⅔.

PLATE XXX.

Fig. 1. Three stages in the collapse of a sporophore on drying. × 1.

Fig. 2. Two later stages of Fig. 1. × 1.

Fig. 3. Slope showing prolific production of sporophores, which vary greatly in diameter. × 1¼.

Fig. 4. Mature sclerotium produced at the tip of a straggling sporophore. × 10.

Fig. 5. Three-weeks-old culture of two monospore strains. The hyphae have mixed, and numerous fusions have taken place, with subsequent production of sporophores. × ⅔.

Fig. 6. Pot of swede seedlings with sporophores which arise from sclerotia beneath the soil surface. The difference in colour and diameter between the stalk and head of the sporophore is well shown. × ½.

Fig. 7. Slope culture with three groups of sclerotia corresponding with bars on the mycelium. × ½.

Fig. 8. Much-branched sporophore from an agar slope. × 2.

Fig. 9. Three-months-old culture of two monospore strains. The mycelia have failed to unite. × ⅔.

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PRODUCTION OF AN ARTIFICIAL EPIDEMIC OF WHEAT STEM RUST IN KENYA COLONY

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(With Plate XXXI.)

There are now known to be four physiologic forms of wheat-stem rust (*Puccinia graminis tritici*) in Kenya. At first two were differentiated by McDonald (1) in 1927 and 1928, later another form appeared on Reliance Wheat at Njoro in 1930, and a fourth form was recognised in 1931 (2). For convenience these physiologic forms are designated by the letter "K," with the suffix 1, 2, 3 or 4. Although in three cases the reactions of these forms on the same differential hosts correspond with those of forms known in North America, it is considered desirable to retain the above method of designating the forms in Kenya. The forms K_1 and K_2 correspond with the American forms 21 and 17 respectively (1), and K_3 gives the same reactions on the differential wheat varieties as form 34 (2).

The work now to be described has been carried out by the writer in co-operation with Mr R. J. Lathbury, Plant Breeder, on the Experimental Station at Njoro. The selection of resistant wheat varieties is always hampered by the fact that only one or two of the four physiologic forms occur naturally in a district in any one year. This difficulty is overcome if the wheats can be exposed in the field to infection from any particular form without depending solely on the natural occurrence of rust forms. At the instigation of Mr G. J. L. Burton, Senior Plant Breeder, attempts were made to produce an artificial rust epidemic in 1932 and in 1933. This was intended to supplement and confirm information which is obtained by means of seedling inoculations; but the knowledge that certain varieties may become more resistant to stem rust in their later stages indicated that observations under field conditions were necessary in addition to the results of seedling inoculations.

The first attempt at producing an artificial epidemic was made in 1932, utilising the forms K_2 and K_4. This was not very successful and only the latter form spread to any extent, but information gained in that year has been utilised in 1933 in the production of an epidemic involving all the four forms. The following account concerns mainly the procedure adopted in 1933, and mention will only be made concerning the previous attempt in so far as it was the cause of different methods being adopted.

METHODS.

The wheat was planted at Njoro in the beginning of May. Several months previously a commencement was made towards working up an ample supply of rust for the time when field inoculations were to begin.

The wheat varieties and hybrids were planted in rows 2 ft. long, twelve seeds being sown by hand in each row; a series of 100 rows occupied a strip 22 yd. long by 2 ft. wide, and was succeeded by a similar strip utilised either as a path, or for planting a "surround" row which was a single drill 22 yd. long.

The plot arrangement may be described as follows:

A = series of 2 ft. rows, 22 yd. long.

B = path 2 ft. wide and 22 yd. long.

C = strip 2 ft. wide and 22 yd. long with a single row of wheat drilled down its length and constituting a "surround row."

These are later referred to under numbers 1–15.

The plot then consists of an arrangement of A, B, and C as follows:

$$A \,.\, B \,.\, A \,.\, C_1 \,.\, A \,.\, B. \, A \,.\, C_2 \,.\, A \,.\, \ldots \,.\, C_{15}.$$

The supply of rust of the four forms was worked up and maintained on wheat grown in pots and boxes inside muslin cages; the latter were similar to those used and described by McDonald (1). In the cases of K_2, K_3, and K_4, there are wheat varieties which are susceptible to one of these rust forms only. By utilising the appropriate variety for multiplying up the physiologic form, it was possible to detect any accidental mixing of forms by the presence of "resistant" pustules.

The following wheat varieties and hybrids were employed:

(The locally bred wheats are referred to by the Senior Plant Breeder in *Annual Reports of the Kenya Department of Agriculture*, 1931, pp. 181–3 and 1932, pp. 145–6, 1933.)

Kenya Governor (local), resistant to K_2 but susceptible to K_1, K_3, and K_4. Used for multiplying K_1.

112 A (local), resistant to all forms except K_2. Used for multiplying K_2.

N.B. 263 (local), same reactions as 112 A.

Einkorn (*T. monococcum*), susceptible to K_2 only.

Reliance, resistant to all forms except K_3. Used for multiplying K_3.

B256 G1A (local), resistant to all forms except K_4. Used for multiplying K_4.

Vernal (Emmer), same reactions as B256.

Marquis, susceptible to all four forms, was also used to some extent for their multiplication.

Seed of these varieties was sown in 6 in. pots and in large boxes (3 ft. long, 1 ft. wide, 8 in. deep). The original supply of inoculum was obtained from the Scott Agricultural Laboratories, where pure cultures of the rust forms are maintained.

The inoculations were carried out as follows. Seedlings were sprayed with water from an atomiser and uredospores of *P. graminis* were applied near the tips of the seedling leaves by means of a flat needle. The plants were again lightly sprayed, and covered with a bell-jar for 48–60 hours. When pustules had developed on the inoculated plants, this rust was transferred to another batch of seedlings. The development of stem sori was encouraged by further re-inoculations as long as the height of the plants allowed of their being covered by the bell-jars. These inoculations were carried out during the dry season of December to February, but owing to the low humidity there appeared to be little natural spread of the rust from inoculated leaves. The inoculations were usually carried out in the mornings, and it was found necessary to remove the bell-jars late in the evening about 60 hours after inoculation. If the plants were uncovered during the day considerable scorching and desiccation of the leaves occurred.

PLANTING IN THE FIELD.

The surround rows were planted on May 11th, and the planting of the wheat varieties in this area commenced on May 15th. As a result of experience gained in the previous year it was decided to plant the "surrounds" some time before the main planting of hybrids and varieties for testing. There is a much better chance of these latter being attacked if they are in the most susceptible stage when the rust has reached its maximum development in the "surrounds." It had actually been intended to plant these rows about 10 days to a fortnight before the hybrids, but owing to the rains being very long delayed the desired lapse in time between the plantings could not be achieved. It appears very desirable that the "surrounds" should be sown at least 10 days before the hybrids in order that the artificial inoculation should be of maximum advantage in providing a severe infection.

In 1932 an attempt was made to establish an artificial epidemic comprising two physiologic forms, K_2 and K_4; the latter developed and spread well, whilst K_2 was present only as a light infection in the surround rows. General information was obtained as to the amount of inoculum material required, and the time necessary to start primary infections at a number of points in the surround rows. It is impracticable to inoculate the whole of the row; the intention is merely to establish the stem rust at a few points, and early enough in the growth of the wheat for there to be time for the rust to spread naturally throughout the row. In certain of the hybrid progenies, information was required as to their reaction to one or two of the rust forms in particular. The wheat varieties were therefore grouped so that the requisite physiologic form could be inoculated on to the adjacent surround row. The various forms ultimately occurred fairly uniformly over the plot area, irrespective of the original points where they were established. The procedure adopted may therefore be considered as satisfactory, and required far less time and inoculum material than if all the rows had been inoculated with the four physiologic forms.

The fifteen surround rows were planted and inoculated as follows:

Row No.	
1–3	B 256 was planted for inoculation with K_4
4–5	112 A was planted for inoculation with K_2
6–9	Marquis was planted for inoculation with all four forms
10–14	112 A was planted for inoculation with K_2
15	Marquis was planted for inoculation with all four forms

It has always been the practice to plant certain tester wheat varieties amongst the hybrids to aid in determining the rust form or forms present. In 1933 four groups of such testers were planted in well-distributed positions. The varieties were not the same in every case but were chosen so as to indicate the rust forms present:

Kenya Governor as a tester for K_1.
112 A or Einkorn as a tester for K_2.
Reliance as a tester for K_3.
B 256 or Vernal as a tester for K_4.

Since Kenya Governor is susceptible to K_3 and K_4 in addition to K_1, it only indicates the presence of the latter form in the field when there is no stem rust present on either Reliance or the K_4 tester.

RUST INOCULATIONS IN THE FIELD.

The inoculations were commenced on June 6th. The inoculum was taken from plants in the muslin cages, either a few rusted leaves were cut off or else the pot containing the plants was carried into the field, and afterwards returned when sufficient uredospores had been transferred.

The method of inoculation was that described previously(1). Seedlings in the surround were sprayed, and the inoculum was applied on a flat needle; the plants were then covered with a bell-jar for about 60 hours. It was always essential to uncover the plants in the evening when the humidity was higher. Brief mention is made by Stakman and Aamodt(3) of methods employed in producing artificial rust epidemics at University Farm, Minnesota; hand inoculations were carried out in rows of susceptible varieties afterwards covered with a bell-jar, and this method appears to be similar to the procedure adopted in Kenya. About 3 weeks after the field inoculations had been commenced, a different method was employed when the plants were of sufficient size. A portion of the row about 2 ft. long was inoculated and the humidity maintained under a cover described here as a "V" glass. Covers of this type were employed in 1932, and are illustrated in Plate XXXI, figs. 1 and 2; they resemble the "cloches" employed in horticulture, but were made locally for convenience and economy. The type of "V" glass employed in 1933 differed slightly from that illustrated in Plate XXXI, figs. 1 and 2. Single sheets of glass were used for the sides instead of three separate panes, as the joins did not permit such a high humidity to be obtained all the time. The dimensions were also increased somewhat, and in the present case were as follows: 3 ft. long, 9 in. wide, 16 in. high.

When the plants had been inoculated and sprayed, three tins filled with water were placed alongside them, and then the whole was covered with a "V" glass. By this means a high humidity was maintained as indicated by a film of moisture inside the glass during even the hottest and driest hours of the day. It will also be realised that the plants received plenty of light, which is of so much importance in rust inoculation work. One advantage of carrying out inoculations under these "V" glasses instead of bell-jars, was that much larger portions of the surround could be inoculated at a time. They were also left on for about 50–60 hours.

The methods described here were successful in producing a severe but localised epidemic of stem rust. Susceptible varieties such as "Marquis" were severely affected within the area of the experiment, but other susceptible wheats a short distance away were only slightly attacked. The presence of the various physiologic forms was observed during the year by noting the occurrence of rust sori on the tester wheats. As explained previously, these varieties can indicate the presence of any of the forms K_2, K_3, or K_4, when occurring together, or the presence of K_1 when K_3 and K_4 are absent. These observations were usually made on nearly mature plants. The variety is considered as showing either resistance or susceptibility according to the occurrence and size of uredosori on the stems. Unfortunately, the appearance of the sori may be affected in various ways by conditions in the field, altering susceptible type sori towards an appearance suggestive of resistance, and *vice versa*. Physiologic forms can only be determined with certainty by inoculating the rust on to seedlings of the differential varieties under controlled conditions. Several samples of stem rust were taken within the plot area and were sent to the Scott Agricultural Laboratories, Nairobi, where the routine testing of rust forms was carried out by Mr F. W. Evans. By means of seedling inoculations under standardised conditions, the presence of all the four physiologic forms within the plot area was confirmed during the year.

No definite information could be obtained as to the part played in the epidemic by stem rust appearing naturally, but judging by the slight amount of stem rust present in wheat fields in the neighbourhood of Njoro, it is probable that there would have been only a very slight infection at the Plant Breeding Station, if there had been no artificial dissemination of the physiologic forms.

REMARKS ON THE ARTIFICIAL INFECTIONS AND SUGGESTIONS FOR THE FUTURE.

It is highly desirable that there should be an interval of at least 10 days between sowing the surrounds and the main planting of varieties and hybrids, since the rust should thereby have reached its maximum development in the surround rows whilst the varieties planted later are still in the critical stage for infection.

It is a great assistance to know whether the desired forms are multiplying naturally in the surrounds, and although the rust appears to develop and spread best on Marquis wheat, it is not possible to tell by observations alone which form is present on this variety, since it is susceptible to all the local forms. It is believed that use might be made of a mixture of seed for planting the surround rows. Thus if an infection with K_2 is required, seed of Marquis and 112 A would be mixed and drilled together for inoculation later. The rust would develop first on the Marquis, being the quicker wheat of the two, and on its spreading to the 112 A this variety would act as a tester and also maintain the rust when the earlier variety was nearing maturity. Several other combinations of wheat varieties could be employed, depending on the number of forms which it was desired to have present in the particular surround row.

The only precaution necessary is that the varieties should be quite distinct in habit when growing together in the row.

SUMMARY.

1. Four physiologic forms of wheat-stem rust are known to occur in Kenya Colony. Only one or two of these forms may occur in any one year, and the work of selecting wheat varieties resistant to several forms is hampered on that account.

2. A method is described whereby an artificial rust epidemic has been produced. The hybrid wheats have thereby been subjected to infection by the four physiologic forms.

3. Certain susceptible varieties were planted as "surround rows" amongst the hybrids, and utilised for inoculations.

4. The inoculum consisted of uredospores, taken from inoculated plants grown in muslin cages, and was applied to leaves of plants in the surround rows. Afterwards these plants were covered for about 50–60 hours with bell-jars or with large "cloches" described as "V" glasses.

5. Suggestions are made as to modifications in the methods of procedure which might be adopted in the production of future artificial field infections in Kenya Colony.

ACKNOWLEDGMENTS.

The writer wishes to thank Mr R. J. Lathbury for his co-operation in the work, and for suggestions and advice on various matters in connection with stem rust from the plant breeder's aspect.

The writer's grateful thanks are due to Mr F. W. Evans, for carrying out the work of testing for the physiologic forms present in numerous samples of stem rust.

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(2) —— (1933). Two new records of physiologic forms of wheat-stem rust in Kenya Colony. *Ibid.* xviii, 218–22.

(3) Stakman, E. C. and Aamodt, O. S. (1924). The effect of fertilisers on the development of stem rust of wheat. *Journ. Agric. Res.* xxvii, 347.

EXPLANATION OF PLATE XXXI.

Fig. 1. "V" glass, end view.
Fig. 2. "V" glass, side view.

(*Received May 25th*, 1934.)

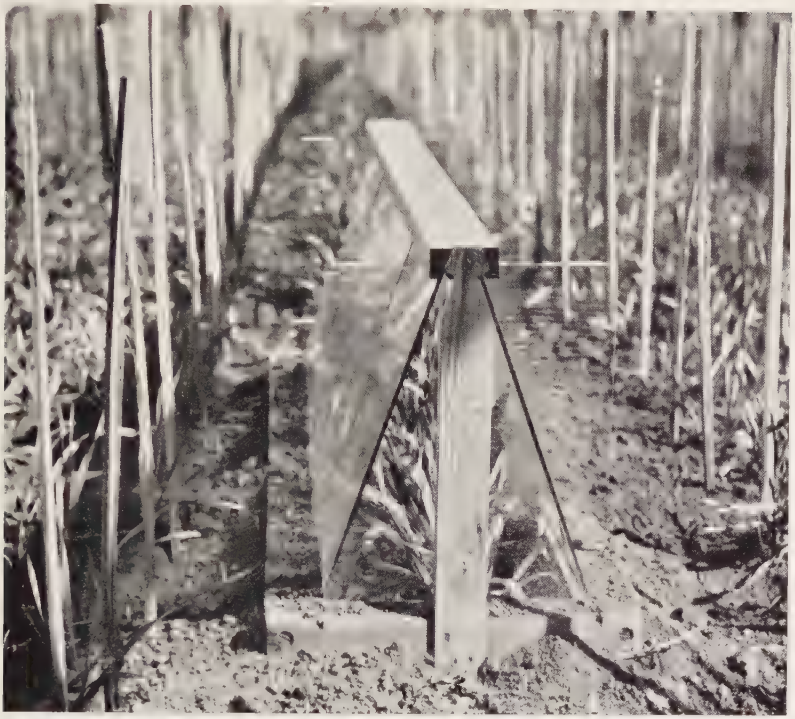

Fig. 1.

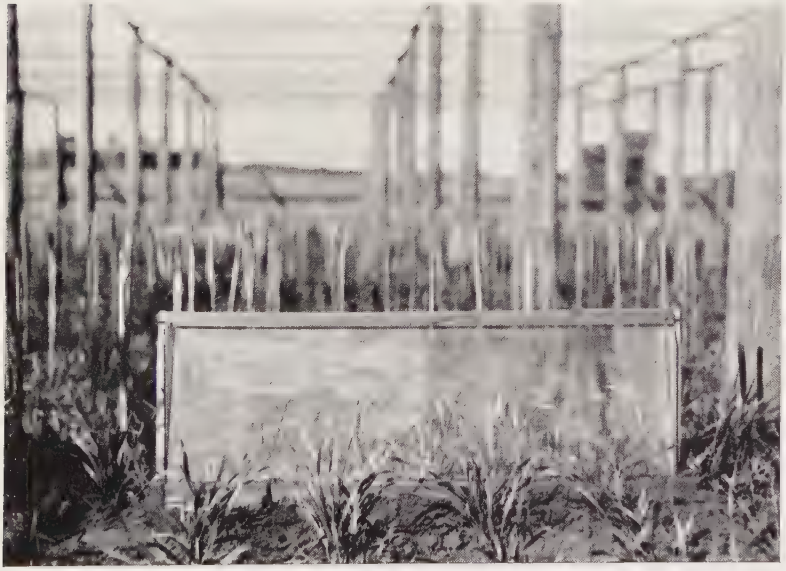

Fig. 2.

THOROLD.—PRODUCTION OF AN ARTIFICIAL EPIDEMIC OF WHEAT STEM RUST IN KENYA COLONY (pp. 614–620).

STUDIES IN BACTERIOSIS

XXI. AN INVESTIGATION OF MARSH SPOT OF PEAS

By MARGARET S. LACEY, M.Sc.

WITH A NOTE ON THE MORPHOLOGICAL STRUCTURE

By BRIAN J. GRIEVE, Ph.D.

(*From the Bacteriological Laboratory of the Imperial College of Science and Technology, London.*)

(With Plate XXXII.)

Introduction.

The disease called marsh spot of peas has been known to exist for many years but little work had been done on the subject until recently when, while the present paper was being prepared, Helena de Bruijn (1) published the results of her investigations on "Kwade Harten" of peas in Holland. A summary of the work of this Dutch investigator and an account of the literature on, and history of, marsh spot is given by G. H. Pethybridge (7) in a paper in the *Journal of the Ministry of Agriculture*, in which he also records the results of some of the work described below.

The name marsh spot was given to this disease because it occurs chiefly in peas grown in low-lying marshy districts, such as Foulness Island in Essex. The disease is severe in Holland, where de Bruijn states that "the damage caused by it in agriculture is very considerable, especially in some instances," and she also states that "the disease is known under the name of 'mouchete' in the Polder land of North France." The present writer also found marsh spot lesions in a sample of pea seeds from California.

Marsh spot of peas is characterised by the presence of reddish brown lesions in the centre of the cotyledons of the seed. The earliest sign of the disease is a slight discoloration of the centre of the inner surface of the cotyledons, varying in shade from pinkish to yellow-buff colour, and affecting only one or two layers of cells. This discoloration gradually spreads through the tissue until in very severe cases practically the whole of both cotyledons may be involved and the cells become reddish brown in colour (see Plate XXXII, fig. 1). In a certain number of seeds the plumule also becomes diseased, and here again there may be only a brown discoloration of the tip or the whole of the plumule may be affected. The browning of the plumule usually accompanies cotyledon lesions, but occasionally seeds have been found in which the tips of the plumules are brown while the cotyledons appear perfectly normal. In most varieties this was an unusual occurrence, but in the crop from No. 680 (see Field Experiments, p. 627), of the 113 diseased seeds examined no less than sixty-three had the plumule affected alone; in thirty-seven cases both plumule and cotyledons were diseased, and in only thirteen seeds was the disease confined to the cotyledons. The microscopical structure and the changes occurring in the tissues in the lesions of the cotyledons and plumules are described by B. J. Grieve at the end of this paper.

The present investigation was undertaken primarily to ascertain whether this disease could be attributed to bacterial action. In addition, observations have been made on the germination and growth of seeds attacked by marsh spot; plants and crops grown from diseased seeds have been examined and compared with those grown from healthy seeds, and finally a little preliminary work has been done on the effect of soil conditions on the development of the disease.

I. THE BACTERIAL INVESTIGATION OF MARSH-SPOT SEEDS.

Cultures on various media have been made from the diseased cotyledons of 200 dried seed peas and 200 green peas taken directly from the pod, in all stages of development and severity of the disease. The peas were taken from samples of a large number of different varieties, "early," "main-crop" and "late," obtained from various localities, including one lot from California[1].

The green peas were removed directly from the pod with sterile forceps and dropped into a sterile dish. The testa was split open and a portion of the brown centres of the cotyledons removed aseptically, crushed and dropped into a test-tube or Petri dish containing a nutrient medium which was incubated for a fortnight at 20–25° C., then left at air temperature for a further period. When dried peas were used they were sterilised externally, then placed on moist filter paper in sterile Petri dishes for 24 hours to swell out, after which they were treated in the same way as the fresh peas. The cultures were made on a variety of media such as broths of a wide range of hydrogen-ion concentration, bouillon agar, pea-extract broth and agar, soil extract, etc., in many cases two or three different kinds of media being inoculated with material from each pea. There was considerable variation in the amount of growth obtained from different samples, but of the total number of peas cultured 60 per cent. of the dried and 80 per cent. of the green peas gave no growth in any media. In several samples (of which that from California was one) the percentage of sterility of the dried peas was as high as 78 per cent.; on the other hand in two cases there was only 30 per cent. sterility. Similarly with the green peas, in cultures made in the summer of 1931 the percentage of sterility varied from 95 per cent. in an early variety (Gradus) to only 56 per cent. in Alderman, but in July 1932 eighteen Alderman peas showing the earliest stage of marsh spot (slight discoloration of the surface of the cotyledons only) gave 100 per cent. sterility. When growth occurred in plate cultures frequently only one or two colonies would appear on each plate, and it was evident from the slow development of turbidity in many of the fluid cultures that here also the number of organisms present in the inoculum was but small. The types of growth obtained in these cultures were grouped as follows: (1) Sporing organisms of *B. mesentericus* or *B. subtilis* type; (2) a minute yellow rod (probably *B. herbicola aureum*, which is commonly found on the exterior of seeds); (3) torulae and cocci; (4) mixed growths of the first three types; (5) bacteria of various species not included in the first three groups (*Bact. fluorescens liquefaciens*, etc.), which were only isolated in one or two cases each; and (6) fungal growths. In some cases all the peas from one sample yielded one species only; for example, of ten seeds of Royal Salute three were sterile and the other seven yielded pure cultures of the small yellow rod, while of nine Stratagem seeds five gave growths of a sporing bacillus and four were sterile. In other cases mixed growths occurred; for example, in cultures made from very badly diseased seeds from Romney Marsh. Of thirty-six of these peas, fifteen gave growths as follows: seven of sporing bacilli, four of the yellow rod, two of torulae, one of a mould and one of an unidentified bacillus.

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[1] The dried seed samples were obtained either from the Cambridge Seed-Testing Station or from Messrs Hurst and Sons.

Table I.

Types of growth obtained in isolation cultures from marsh-spot peas.

Type of growth	Dried pea cultures %	Green pea cultures %
(1) Sporing bacilli	29	22·5
(2) Yellow rod	30	15
(3) Torulae and cocci	7·5	10
(4) Mixed growths	12·5	15
(5) Other bacterial types	15	25
(6) Fungi	6	12·5

The total percentages of the different types of growth obtained from all cultures are given in Table I.

The Table shows that the two predominating organisms infesting marsh-spot seeds are the sporing type and the yellow rod, which were found in equal proportions in the dried seeds. These two types, however, may also be present in seeds free from marsh spot. Thus cultures were made from the centres of cotyledons of 60 peas taken from samples entirely free from marsh spot. Growth occurred in five of these, four being of the sporing type and the fifth the yellow rod. Also, if healthy peas are kept on moist filter paper in sterile Petri dishes for several days, quite commonly a film of either white or yellow growth will develop on the exterior, and both the sporing and the yellow types were found covering the exterior of peas in some pods which had become saturated with water. The cotyledons in all these cases were entirely unaffected. It therefore appears that these types are normally present as saprophytes in pods and on the exterior of seeds, and the higher percentage of growth obtained from the interior of marsh-spot peas is probably due to the favourable conditions for saprophytic growth produced by the dead tissue of the cotyledon lesions. Further proof of the saprophytic nature of the bacteria obtained in these isolations is afforded by inoculation experiments. Cultures of all the types isolated have been inoculated into young pea seedlings and also into pods in various stages of development, invariably with negative results. Moreover, if the disease was of bacterial origin the organisms should be present in large numbers in the early stages in young green peas, but this is not the case. For example, of cultures made from young lesions of sixty green peas of Gradus variety (taken directly from the pod immediately after picking) only three developed bacterial growth, and, as stated above, cultures made from eighteen Alderman peas showing the earliest signs of marsh spot were all sterile. This absence of bacteria in the young lesions has been confirmed by numerous direct microscopical examinations of fresh tissue and also by microtome sections made by Dr Grieve and stained by methods commonly employed to demonstrate bacteria in tissues.

All the evidence obtained by cultural and inoculation methods is therefore against the bacterial origin of this disease.

In order to determine whether any living agent or virus was the cause of marsh spot, both seedlings and pods of various ages were inoculated with macerated diseased tissue and with extracts of this passed through a bacterial filter. In every case corresponding extracts of healthy peas of the same variety were used for control inoculations.

The seedlings showed no ill effects from the inoculation of either healthy or diseased tissue emulsions. The first trials of pod inoculations made in 1929, with unfiltered extracts, appeared to a very slight extent to favour a virus origin of the disease, for, although negative results were obtained from all inoculations made in June, in a further series done on July 18th three out of the thirty-six pods inoculated with diseased material contained marsh-spot seeds, giving a total of five infected seeds out of 103. No peas were affected either in the control pods or in many other untreated pods in the same rows examined at the same time. These inoculations were therefore repeated in 1931 with the following results. Inoculations into Meteor pods were invariably negative, but the following percentages of marsh spot occurred in peas in inoculated pods of Alderman: none in pods inoculated with unfiltered control extract; 16 per cent. from filtered control extract; 14 per cent. from unfiltered, and 15 per cent. from filtered, diseased extract. (The percentage of marsh spot occurring in untreated pods of Alderman in this plot was 4 per cent.) Pods on the same plants were used as far as possible for the inoculation of both diseased and healthy extracts and for the examination of untreated seeds. As syringe inoculations of about $\frac{1}{2}$ c.c. were made through the ventral suture at the stem end of the pod, the increase in marsh spot in inoculated pods might suggest some metabolic or mechanical disturbance in the developing pod as the cause of the disease; the lack of marsh spot, however, in the unfiltered control series is evidence against this theory. Further tests with the extracts during the following year were useless, since a fair percentage of marsh spot developed in the rows grown for inoculation purposes.

II. THE GERMINATION AND GROWTH OF SEEDS INFECTED WITH MARSH SPOT.

(*a*) *Germination in sterile sand.*

Seeds from samples of marsh-spot peas, either with or without surface sterilisation with mercuric chloride, were placed on moist filter paper for 48 hours; the testa was then split and the cotyledons gently prised apart and examined. The seeds were separated into four groups: those with (*a*) unmarked cotyledons, (*b*) very slight discoloration only, (*c*) considerable discoloration, and (*d*) very severe discoloration, the greater part of both cotyledons and frequently the tip of the plumule being involved. The seeds were placed in sterile sand to germinate. No difference was apparent between the seedlings raised from the groups (*a*) and (*b*), but seeds in group (*d*) usually failed to germinate, while those of (*c*) germinated normally, though occasionally the primary shoot died soon after. This death of the plumule, followed in most cases by the development of laterals which were quite healthy and which produced normal plants, frequently occurred in seedlings from marsh-spot seed both in pot and in field experiments, but it has also been occasionally observed in those grown from healthy seed. In both cases the primary shoot would grow to about 1 in. above ground but fail to develop further, it usually had a club-shaped tip, and, after laterals had formed, would turn brown and die (see Plate XXXII, fig. 2). In some seeds the plumule shows the same discoloration as the cotyledons, and it is probably usually from such seeds that these diseased primary shoots are produced. Also, in badly diseased seeds the growth of saprophytic organisms in the dead tissue may proceed after planting to such an extent that the embryo is injured. Occasionally in the examination of the diseased cotyledons of marsh-spot seed, some were found with deep-rotted lesions which contained numerous bacteria; and the seeds of group (*d*) which failed to germinate were quickly rotted by bacterial action. That this was a secondary infection following the true marsh-spot lesion is shown by the fact that these bacteria were found only in very advanced stages of the disease, and that inoculations with the mixed growths found in such lesions gave negative results.

(*b*) *Germination and growth in pot cultures.*

In the spring of 1929 seeds from the 1928 field trials of varieties Nos. 680 and 669 (see p. 628) were examined as in the sterile sand experiments, separated into (A) healthy and (B) marsh spot (none of which were of the very severe type which fails to germinate), and thirty of each planted in pots of autoclaved soil in a greenhouse. All the seeds germinated and the (A) lot developed normal seedlings, with the exception of two in which the main shoots died when about 4 in. high and were replaced by lateral growth. Of the (B) seedlings seventeen were normal, nine had diseased plumules which died, the growth being continued by side shoots, and four seedlings were very unhealthy with brown streaks or spots on the shoots. Two of these plants died a little later; the other two developed healthy side shoots. After this initial trouble, when the lateral shoots had compensated for the death of the main shoot, the plants of (A) and (B) were similar in development, and flowered at the same time. The greenhouse conditions were not favourable for the setting of seed, but twenty-seven pods developed on the (A) plants and twenty-four on those of (B), all of which contained perfectly healthy seed.

In a further experiment in 1932 two lots of Laxton's Superb pea seed, one severely infected with marsh spot, and the other free from disease, were examined for the presence of lesions in the cotyledons as in the former trials. Of the marsh-spot lot 120 seeds were selected which showed definite lesions in the cotyledons and in many cases discoloration of the plumule also, but very badly diseased seeds like those of (*d*) in the sterile sand experiments were rejected, as these usually failed to germinate. These 120 marsh-spot seeds, together with 120 healthy seeds, were planted in pots of soil which had been sterilised in the autoclave for 6 hours at 20 lb. pressure. The marsh-spot seeds were somewhat slower in germinating than the healthy seeds, but finally 105 of the former appeared above the soil against ninety-nine of the latter. Of these 105, however, no less than sixty-three had diseased "club-head" primary shoots, but not one of the healthy control seedlings was affected in this way. In consequence, for the first 3 weeks of growth there was a marked difference between the control plants, which were strong and sturdy-looking, and the marsh-spot seedlings. After this period, however, the latter plants grew so well that in 6 weeks no difference could be detected between the plants in the two lots in general appearance and vigour. All the seeds from these plants were entirely free from marsh spot.

(*c*) *Field experiments.*

In 1928 rows of marsh-spot seed of varieties Pilot, Thomas Laxton, Alderman and two unknown varieties (Nos. 669 and 680) were planted in the Chelsea Physic Gardens with, in the first three cases, control rows of healthy seed of the same varieties in an adjacent bed. For some reason the germination in all was very poor, but was considerably lower in the marsh-spot rows as Table II shows:

Table II.

		% germinated	
Date planted	Variety	Clean seed	Marsh-spot seed
26. iii. 28	Pilot	73	57·5
10. v. 28	Pilot	75	47
10. v. 28	Alderman	52	15
26. iii. 28	Thomas Laxton	50	37
10. v. 28	Thomas Laxton	48	38
26. iii. 28	No. 669	—	49
26. iii. 28	No. 680	—	45

Of the young seedlings, 27 per cent. of Thomas Laxton from marsh-spot seed had diseased plumules, but none of the control seedlings. This was reversed in the first planting of Pilot in which there were 12·5 per cent. diseased primary shoots in the control row and only 4·3 per cent. in the marsh-spot row, while both rows of the second planting were unaffected. Both Alderman rows showed very poor growth, and on the whole, in all the varieties, the plants grown from marsh-spot seed were somewhat inferior to those grown from healthy seed. This was the only year in which any difference in the later growth of the plants could be detected. Two plants in each of the No. 669 and No. 680 rows had mosaic disease; they were stunted, with distorted mottled lesions and pods streaked with yellow and with a warted appearance. There was, however, no sign of marsh spot in any of the seeds from these two plants, though the disease was found in seeds from apparently perfectly healthy plants in the same rows. (The crop from this series is dealt with later, see (3).)

In April 1929, seeds from the 1928 crop of No. 669 were germinated on filter paper, the cotyledons examined for the presence of lesions and (A) forty seeds with no sign of disease, and (B) forty seeds badly affected with marsh spot planted in adjacent rows at Chelsea. Thirty-one A and thirty-four B developed, of which eight of the latter had diseased plumules but none of the former. After this appearance in the young seedlings, no difference at all in the later growth could be detected between the plants in the two rows. The season of 1929 was apparently unsuitable for the development of marsh spot, and no seed could be obtained for experimental purposes in 1930; but the 1930 crop from various parts of the country was badly diseased and numerous samples were obtained from the Cambridge Seed-Testing Station. In the spring of 1931, therefore, the experiment of 1929 was repeated, peas of varieties Meteor, Gradus, and Alderman being germinated, examined for marsh spot, separated into (A) healthy and (B) diseased seed and planted in adjacent rows. Control rows (C) of healthy seed of these three varieties were also planted. All the rows A, B and C of each variety germinated equally well, and no difference could be detected at any time between the plants of the three lots in the Meteor and Gradus series. Alderman B rows had 16 per cent. diseased plumules, and both A and B of this variety had a few unhealthy seedlings with distorted yellowish leaves, which were not seen in the C row. Later, however, all the plants appeared perfectly healthy and, two months after planting, the Alderman A and B rows looked somewhat stronger plants than those of row C. In addition to the trials in the Chelsea Physic Gardens, rows of marsh spot and healthy seeds of Alderman variety were planted at Slough, at the Imperial College Field Station, with results similar to those obtained at Chelsea. After the initial seedling stage no difference could be detected between the plants.

Further trials were made in 1932, the varieties used being Freedom and Alderman, and, as before, the cotyledons were examined before planting to ensure that all the seeds planted in the marsh-spot rows were infected and all those in the control rows were healthy. Results similar to those of the preceding year were obtained. In the Freedom series, seventy-seven out of 100 germinated in the marsh-spot rows, of which ten had diseased plumules, while eighty-three of the 100 healthy seeds germinated and all were normal. Ninety-three out of 200 Alderman marsh-spot seeds germinated and 114 of the healthy seeds; each row had two seedlings with diseased plumules. No difference in the subsequent growth of the diseased and healthy rows of either variety could be detected. A few plants of each variety appeared to have mosaic disease; the seeds of these plants were free from marsh spot. In all these trials the plants were carefully examined for any signs of bacterial disease, but no stem or leaf lesions, streaking or spotting, etc., could be detected in the plants grown from marsh-spot seed which did not similarly affect those grown from healthy seed. Lesions due to *Ascochyta* were sometimes found, and in one case a heavy infection of *Bact. pisi* occurred on some plants grown from seeds free from marsh spot, but the seeds of the plants attacked by these pathogens did not develop marsh spot.

The results of these experiments indicate that, except that there is in some cases a lower germination of marsh-spot seeds and frequently death of the plumule followed by an abnormal development of young seedlings, the disease has no effect in most seasons on the subsequent growth and health of the plants.

III. THE COMPARISON OF THE CROPS OF PLANTS GROWN FROM MARSH-SPOT AND HEALTHY SEEDS.

There is very little evidence of any decrease in the yield of plants grown from marsh-spot seed. In 1928 the total yield from the healthy seed of each variety was considerably greater than that from the marsh-spot seed, but this was due to the lower germination of the latter. The average yield per plant was practically the same in both healthy and marsh-spot rows, with the exception of the first sowings of Pilot, in which the yield from the healthy plants was double that from the marsh spot; the second sowings of Pilot seed, however, gave identical yields. In the 1929 series of No. 669 the average yield per plant was very slightly more from the healthy seeds than from the marsh-spot seeds, but in 1931 and 1932 the average yields from the healthy and marsh-spot seeds of every variety tested were practically identical.

Most of the pods on the plants grown in these trials were picked and the seeds examined for the presence of marsh spot. Table III gives a summary of the results obtained; the percentage of disease present in the original seeds planted in the marsh-spot rows is given in the third column.

Table III.

The development of marsh spot in the crops of plants grown from healthy and diseased seed.

Year	Variety	Percentage of disease in seeds planted in marsh-spot rows	Percentage of marsh-spot seed in the crop		
			From marsh-spot seed		From healthy seed
1928	Pilot, 1st sowing	20	0		0
	Pilot, 2nd sowing	20	3·6		1·7
	Laxton, 1st sowing	25	0		0
	Laxton, 2nd sowing	25	0·6		3·5
	Alderman	33	17·5		6·3
	No. 669	35	12·5		—
	No. 680	33	25		—
1931	Alderman at Slough	70	30		29
1932	Freedom	100	4		12·5
	Alderman	100	27		21
			A Marsh-spot seed	B Clean seed from same lot as A	
1929	No. 669, 1928 crop	100	1	0	
1931	Meteor	100	0	0	0
	Gradus	100	14	11·5	6
	Alderman	100	4	3·8	4

Table III shows no evidence of any seed transmission of the disease; in some cases there was a slightly higher percentage of marsh spot in the crop from the diseased seed, but in others there was as much marsh spot in the progeny from healthy seed as from diseased seed, while in Freedom of 1932 12·5 per cent. of the crop from the healthy seed was infected and only 4 per cent. of that from the marsh spot. The early sowings of Pilot and Thomas Laxton in 1928 and Meteor of 1931 yielded perfectly healthy crops from both healthy and marsh-spot seed. The 1928 results are open to the criticism that, as the marsh-spot samples contained a good proportion of clean seed, it may have been mainly from these that the new crop was produced. This does not apply, however, to the later trials at Chelsea Physic Gardens, for in these each seed was examined before planting and only those showing definite cotyledon lesions were included in the marsh-spot rows.

The figures suggest that the conditions (? soil) at the Chelsea Physic Gardens are not favourable for the development of the disease. Thus, the original sample of No. 669 contained 35 per cent. of marsh-spot seed, the crop from this only 12·5 per cent., while the 1929 crop grown from the latter had only five infected seeds out of 442 of those examined from the marsh-spot row and none in the control row. The same result is apparent in the 1931 trials of the Alderman variety. The original seed was 70 per cent. infected by marsh spot, but only 4 per cent. of the progeny grown at Chelsea Physic Gardens was diseased. The percentage (30) was higher in the crop from seed of the same sample grown at Slough, but this again is considerably less than the original, and the healthy row was just as much affected.

There is some evidence that the disease appears late in the season and is worse in late varieties than in the early kinds. In the seed samples examined there was generally a higher proportion of infected seed, and the cotyledon lesions were more extensive in main crop and late varieties such as Alderman and Freedom than in early kinds like Meteor and Pilot. Also in the 1928 trials, as shown in Table III, the crops from the sowings of Pilot and Thomas Laxton, made in March, were quite free from marsh spot, but there was a small proportion of infected seed from the second sowing made on May 10th in both; and in the same year No. 669 and No. 680, both of which were late varieties, gave a considerably higher percentage of infected seed than the earlier varieties. Similarly, in 1931 Meteor gave an entirely clean crop; the first seeds of Gradus picked on June 25th were unmarked, but those examined on July 14th–21st had some marsh-spot peas in all three rows, and the same occurred with the Alderman rows. In 1932 pods from the Alderman rows were examined at weekly intervals. Those picked on July 12th and 19th were free from marsh spot; on July 28th 25 per cent. of the seeds from the marsh-spot row and 17 per cent. of those from the healthy row had a slight pinkish brown discoloration of the cotyledons, and one pea had a plumule completely browned as well as fairly deep cotyledon lesions. A week later 39 per cent. of the seed from the marsh spot and 32 per cent. of the control rows were affected; in most of these the disease was still slight, but some had very extensive cotyledon and plumule lesions. The pods picked on August 8th had a smaller proportion of diseased seed (25 per cent. in the marsh-spot and 17 per cent. in the control rows), and again all stages of the disease were found. The seeds examined each week were as far as possible of about the same age. Examinations of numbers of seeds have shown that marsh spot does not appear until the pea is fully developed, and usually a little past the age at which it would be picked for eating, but the disease may develop very severely in the green peas, and further ripening does not appear to enhance the severity of the attack.

Distribution of marsh spot seeds in pods and on plants.

There is, apparently, no regularity in the distribution of marsh-spot seeds in a pod or of infected pods on a plant; both diseased and healthy seeds are found in the same pod and diseased and healthy pods on the same plant. Numerous pods have been examined in order to determine whether there was any evidence of an infective agent entering from either the stigma or the stem end of the pod, but no indication of such an occurrence could be detected. Pods were found containing, for example, one diseased and one healthy seed, six healthy and one in the centre diseased, six diseased and one healthy, etc., and the marsh-spot peas might be either at the stigma or the stem end, in the centre or alternating with the healthy seed. In 1931 sixty plants of Alderman were examined individually, and of these thirty-two bore entirely clean seed. The remaining twenty-eight had a varying number of pods infected, and these were found in diverse positions on the plant. For example, on one plant the lowest pod contained three clean seeds, the second pod five clean and three marsh spot, the third one clean and four marsh spot, the fourth eight clean and one marsh spot, while the fifth and sixth pods bore only healthy seeds. On another plant the two lowest and the two top pods contained only healthy seeds, but the middle one had seven marsh-spotted seeds; other plants had the lowest affected and the upper clean or *vice versa*. The extent of the lesions also varied on peas in the same pod; peas having the merest trace of discoloration on the surface of the cotyledons were found side by side with those having the whole of the centres of both cotyledons, and sometimes the plumule also involved. It was not possible to diagnose the presence of marsh spot from the appearance of the pod, or the exterior of the peas; often the largest and best-looking pods would contain very badly diseased seeds, which outwardly looked perfectly sound.

IV. EXPERIMENT ON THE EFFECT OF SOIL AND PHYSIOLOGICAL CONDITIONS ON THE DEVELOPMENT OF MARSH SPOT.

In 1931 through the kindness of Dr Tincker, of the Royal Horticultural Society's Gardens, Wisley, a number of seeds from plants (variety Little Marvel) grown in the gardens on four types of soil—sand, clay, loam and chalk—were examined for marsh spot. The seed from the first three was entirely free from marsh spot, but five of the seventy-eight pods from the chalk contained some marsh-spot seed, giving a total of fifteen peas out of 364. This is a very small percentage, but was an unexpected result in view of the common occurrence of the disease in marshy districts.

In the spring of 1932 an experiment was undertaken to test the effect of soil on the production of marsh spot. A quantity of soil from Foulness Island in Essex from land which had repeatedly borne severely marsh-spotted crops, was obtained through the kindness of Mr Newby, of Hurst and Sons. The soil was an alluvial clay, containing numerous pieces of shell, with a hydrogen-ion concentration of 6·9. Wisley sand was used as a control, for, besides its being a different type, Dr Tincker states that he has seen no marsh spot in peas grown on this soil, and this was confirmed by the examination of a number of seeds of the 1931 crop. The two soils were placed in 12 in. pots, some of which were then sterilised in the autoclave. Healthy Meteor and healthy and marsh-spot Freedom seed were each planted in pots of soil of the following four types: (*a*) untreated Foulness soil, (*b*) sterilised Foulness soil, (*c*) untreated Wisley soil, and (*d*) sterilised Wisley soil. The Freedom seed was germinated and examined before planting, and only those seeds bearing definite lesions were used in the marsh-spot series. (The marsh-spot Freedom seed came from plants grown at Foulness the previous season.) It was thought that, if the disease was physiological and due to soil conditions alone, all the plants grown in Foulness soil, untreated or sterilised, should have yielded some marsh-spotted seed. If there was an infective agent in the soil, the sterilised soil should have yielded clean crops, at any rate from the healthy seed, while it was hoped that further evidence of any seed transmission of the disease would be obtained. The result, however, was disappointing, as marsh spot was entirely absent in the crops throughout the series.

The seedling growth in this series was similar to that obtained in previous pot experiments, the healthy seeds of Meteor and Freedom giving rise to normal seedlings, while 28 per cent. of the Freedom marsh-spot seedlings had diseased primary shoots with later development of healthy laterals. All the plants grown in the Foulness autoclaved soil were extremely poor and unhealthy, but the sterilisation of the Wisley soil had practically no deleterious effect on the growth of the plants. A culture of *Bact. radicicola* was watered into the soil of all the pots a month after the plants had appeared above ground, as it was thought that the absence of this organism in the sterilised soil might partially account for the poor growth; but no beneficial results were apparent from this inoculation. The best plants were those grown in the Foulness unsterilised soil, and, after the seedling stage, the plants from marsh-spot seed in this soil were quite as good as those from healthy seed.

In 1933 further pot experiments were set up to test the effect of varying the water content of the soil and of atmospheric conditions. The varieties of peas used were Sherwood and Early Marvel, both dwarf varieties suitable for pot experiments. Twelve per cent. of the Sherwood seed was infected with marsh spot, so that this variety was known to be susceptible. The soil used was either from Foulness, Wisley or Slough, pots of each being treated by the various methods. These methods included: (*a*) Standing the pots in deep dishes of water, so that the soil was waterlogged, while control pots were kept normally watered. Some of each of these were covered with bell-jars so that the plants were kept in moist air, others were left uncovered, and some were placed in a greenhouse, others in the open air. (*b*) Alternate soaking and drying, the soil being kept waterlogged for 2 or 3 days, then allowed to become very dry, then soaked again and so on. (*c*) Alternate exposure to saturated and dry air; the plants being first covered with a bell-jar after watering, then exposed to a hot sun. In addition some plants were kept constantly in the shade, and others in the sun, with or without excessive watering. As in 1932 the result of all these experiments was entirely negative; no marsh spot developed in any of the seed from any of the plants. Rows of the same varieties grown in the open ground in Chelsea Physic Gardens were also almost free from marsh spot, 2·5 per cent. of Sherwood and 1·3 per cent. of Early Marvel only being affected, but, owing to the very dry conditions, these plants and crops were very poor.

The only conclusions to be drawn from these two years' experiments are that pot cultures do not provide the right conditions for the development of marsh spot, and that the type of soil is not the only factor, nor excessive watering[1].

DISCUSSION.

The results of these investigations agree with those obtained in Holland by H. A. A. van der Lek (4) in 1918 and recently by Helena de Bruijn (1). These workers both concluded that neither fungi nor bacteria were responsible for the disease, and de Bruijn (1) considers that the trouble is physiological in origin, although she was not able to determine the actual cause. They observed defective seedlings arising from diseased seeds similar to those described in the present paper and also concluded that the disease was not transmitted by seed. G. H. Pethybridge (7) described some experiments on the germination and growth of marsh-spot pea seed carried out at the Plant Pathological Laboratory at Harpenden and by Mr Brett at the Cambridge Seed-Testing Station with results similar to those obtained by the writer. Both Pethybridge (7) and de Bruijn (1) compare marsh spot with a bacterial disease of peas described by D. M. Cayley (2), and they each consider that, although some of the symptoms of the latter disease are similar to those of marsh spot, yet there are essential differences between the two diseases. De Bruijn (1) thinks it possible that Cayley was working simultaneously with two different diseases, and the present writer is also of this opinion.

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[1] De Bruijn also obtained mostly negative results in pot experiments, and attributes the failure to the fact that it is difficult by this method to obtain very vigorous plants, without which, she says, marsh spot cannot be found.

Marsh spot appears to have a more serious effect in Holland than in England, but in both countries the extent of the trouble varies considerably in different seasons. The amount of damage caused by sowing marsh-spot seed is also variable. Probably no ill effects at all will follow the sowing of seed which is only slightly diseased, but when a high percentage is badly affected a considerably lowered germination may occur and many of the seedlings will have diseased primary shoots. Here again the later development of such seedlings will vary. Under favourable conditions the plants will outgrow the disease and no further trouble will occur, but under unfavourable conditions (very wet soil for example) the weakened seedlings are more easily attacked by moulds, etc., before the lateral growth has had time to compensate for the death of the primary shoot.

SUMMARY.

1. A bacterial examination was made of the cotyledon lesions of 200 dried and 200 fresh green-pea seeds. Cultures made from 60 per cent. of the dried and 80 per cent. of the fresh peas were sterile; the remainder yielded growths of micro-organisms of which the two predominating species were a sporing bacillus of the *B. subtilis* or *B. mesentericus* type and a minute yellow rod, probably *B. herbicola aureum*.

2. These two types are often present on the outside of pea seeds, and were isolated in a few cases from the centres of the cotyledons of healthy peas.

3. Further proof of the saprophytic nature of these two types, and of the other organisms isolated in the cultural tests, was afforded by numerous inoculation experiments, which all gave negative results.

4. Tests on the germination of marsh-spot seeds show that badly diseased seeds fail to germinate, while in less severe cases the primary shoot is frequently dead or dies soon after germination, the growth being continued by lateral development.

5. After the seedling stage in most seasons no difference can be detected between plants grown from marsh-spot or healthy seeds.

6. No evidence was obtained of any increase of marsh spot in the progeny from diseased seeds as compared with that from healthy seeds grown under the same conditions.

7. Pot-culture experiments on the effect of soil and of excessive watering on the development of marsh spot gave entirely negative results. Pot cultures are apparently unsuitable for the development of marsh spot.

In conclusion I wish to express my gratitude to the Cambridge Seed-Testing Station for supplying samples of seed; to Mr Newby, of Hurst and Son, for seed samples and also for obtaining the soil from Foulness; and to Mr Hales for his care of the plants grown in the Chelsea Physic Gardens. I am indebted to Dr Pethybridge for the loan of a translation of Miss de Bruijn's paper and for the opportunity to read his paper on marsh spot before publication. Finally, I wish to thank Dr S. G. Paine for his advice given throughout the course of this investigation.

NOTE ON THE MICROSCOPICAL INVESTIGATION OF MARSH SPOT OF PEAS.

By BRIAN J. GRIEVE, Ph.D.

THE MICROSCOPIC STRUCTURE OF MARSH-SPOT PEAS.

Technique. Both fresh and dried diseased seeds were used in the investigation. In the latter case they were of course swelled before examination. Some of the material was examined in hand-cut sections, but most in microtome sections after fixation in medium chrom-acetic.

Sections down to 5μ in thickness were obtained. Stoughton's Thionin-Orange G stain (8) was employed in staining the sections to determine whether bacteria were present in the necrotic lesions. Numerous microtome sections of cotyledons showing various stages of disease from small necrotic specks to large blotches failed to show the presence of any bacterial organism. These results suggest that the disease lesions with which Cayley (2) worked (and which from her photos are indistinguishable from the ones under observation) may have represented the cotyledon reaction of a separate bacterial disease. The presence of bacteria in the cells and intercellular spaces as she figures them could not be demonstrated in the marsh-spot lesions with which we were working. Sections were also made of the browned tips of the plumules; here again no bacteria could be demonstrated. Having settled this point a study was made of the microscopic structure of the lesions, both hand-razor sections and microtome sections being used.

Although in macroscopic examination the necrotic tissues in the cotyledons seemed well defined in relation to the healthy tissues, in thin razor sections under the microscope the line of junction could not easily be detected. There is no wound reaction at the junction of necrotic and healthy cells. No cork layers are formed as is the case in macroscopically similar lesions in potatoes affected with Spraing disease. On first examination it appears that the starch grains remain in diseased cells to a greater extent than they do in healthy cells. This, however, is not really the case; the effect is produced by the disappearance of most of the cytoplasm in the necrotic cells leaving only the starch grains, while in the healthy cells the starch grains are not so noticeably embedded as they are in the dense cytoplasm. The starch grains while remaining in place in the diseased cells do not appear to have any definite membrane surrounding them and acting as a support. On dissolving out the starch grains with caustic potash no trace of any membrane such as surrounds the grains in the Spraing and Pseudonetnecrosis diseases of potato tubers was seen (Kerling(3)). The accompanying camera lucida drawing of a necrotic area in a cotyledon illustrates the various points mentioned above (Plate XXXII, fig. 3). Sections of the browned plumules showed that in some cases the necrosed areas extended into the apical bud region. Frequently only the outer bracts showed browning of the cells. These browned cells were quite clearly to be distinguished from the apparently healthy cells in the plumule and the line of junction was quite clear even in unstained sections. Here as in the cotyledon there was, however, no indication of the formation of a cork cambium between the healthy and necrotic cells.

Micro-chemical tests.

The following tests were carried out in an endeavour to throw some light on the nature of the chemical changes which had occurred in the lesions of the cotyledons and in the browned tips of the plumules. Thin hand-razor sections of the cotyledons and plumules were used. These were subjected to the tests in small reagent dishes and then examined under the microscope.

Suberin.

Potash solution. Necrotic cells stained a deep yellow with 3 per cent. potash after being exposed to this reagent for some hours. The yellow colour in the affected cell walls was apparent in a short time when concentrated potash solution was used. The suberin-containing cell walls of the necrosed area are clearly marked off from the non-stained walls of the surrounding healthy cells. In the drawing reproduced here (Plate XXXII, fig. 4) the shaded portion represents the cells stained yellow with caustic potash. In sections cut at right angles to the adjacent inner faces of the cotyledons, and treated with potash, it was to be seen that in some cases even the epidermal row of cells was stained yellow, indicating the presence of suberin there. Frequently, however, the epidermal row of cells did not show any stain (Plate XXXII, fig. 5). The above tests show that suberin is present in the necrotic cell walls. The factors governing the spread of the toxic substances associated with the formation of the lesions are not clear. Neither is it known why the central parts of the cotyledons constitute the centre from which the toxic substances emanate.

Sudan III. The presence of suberin in the diseased cell walls was further shown by the use of Sudan III. When sections were treated with this reagent the walls of the necrotic cells stained reddish brown. Necrotic cells, however, were not so clearly delimited from healthy surrounding cells.

Lignin.

Phloroglucin and hydrochloric acid. A weak reddish coloration was found to occur in the walls of affected cells. Suberised walls, however, can give wood reactions, so that this result is not conclusive as to the presence of lignin.

Pectic substances.

Ruthenium red. In sections stained for a few seconds with ruthenium red, 1 in 1000 dilution, the necrotic cell groups showed a much stronger affinity for the stain than healthy cells. When sections were stained longer, however, the whole of the tissues showed a red coloration. It appears from the following that the pectic content in the diseased cell walls is an insoluble pectic acid. After staining with ruthenium red the sections were treated with acid alcohol (1 part HCl, 4 parts alcohol) for 10 min., then washed and restained. Microscopical examination showed that the red stain had been taken up by the necrotic cell walls, while neighbouring healthy cell walls showed no definite coloration. Some showed a pale brownish colour. Since, according to Mangin (5), acid alcohol dissolves pectose and other soluble pectic compounds, leaving the insoluble pectic acid, it would appear from this test that insoluble pectic acid is present in the diseased cell walls. The first test with ruthenium red indicated the presence of gums and mucilages derived from pectic compounds, since Mangin (6) has also found that ruthenium red acts on such compounds without staining those derived from cellulose or callose.

A further test demonstrating the presence of pectic substances in the necrotic cell walls was carried out as follows: Sections were treated with cuprammonia, thus dissolving out the cellulose, and then stained with ruthenium red. The sections showed a red stain throughout, but the coloration was stronger in the cell walls of the disease area. The central parts of the necrotic cell groups were yellowish brown in colour after treatment with cuprammonia, and did not show any appreciable change from this on treatment with the ruthenium red.

Methylene blue. This test also indicated the presence of insoluble pectic compounds in the necrotic cell walls. The stain was taken up by all the cells when used alone in a dilution of 1 in 1000. The necrotic cells, however, showed a more greenish colour in contrast to the blue of the healthy cell walls. When sections were stained after being treated with acid alcohol the stain was only taken up in the necrotic cell complex. Here the colour of the necrotic cell walls appeared definitely green.

Solubility.

After boiling in 3 per cent. KOH for 10 min. the tissues had to a large extent disintegrated. It was, however, not easy to define the actual necrotic cells after boiling, as that operation had caused the disappearance of the yellow colour. There appeared to be no marked difference in any case in the degree of resistance between the healthy and necrotic cell groups. On placing in strong sulphuric acid the diseased parts of the tissue turned a deep brown colour. Healthy tissues were rapidly disintegrated and the necrotic tissues also dissolved in a few hours. Both healthy and necrotic tissues were rapidly dissolved when placed in 50 per cent. chromic acid. Sections treated with strong hydrochloric acid, however, showed no obvious change even after several days. From these results it appears that the necrotic cell groups do not possess any great degree of resistance over that of normal tissues.

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EXPLANATION OF PLATE XXXII.

Fig. 1. Cotyledonary symptoms of the marsh-spot disease of peas.

Fig. 2. Pea seedling showing the diseased plumule and the development of a healthy lateral shoot.

Fig. 3. Camera lucida drawing of part of a necrosed area in a pea cotyledon. Note the absence of any wound cork at the junction of the necrotic and healthy cells.

Fig. 4. Transverse section of a pea cotyledon with necrotic suberin-containing cell walls shaded in black.

Fig. 5. Longitudinal section of a pea cotyledon to show the relation of the diseased suberin containing cells to the surface.

(*Received May 3rd*, 1934.)

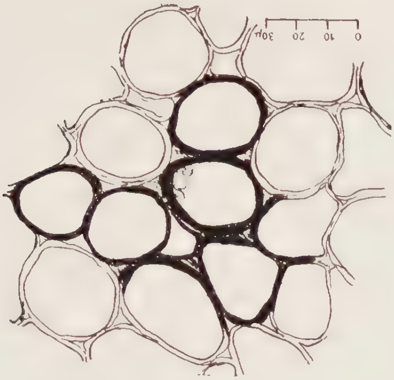

Fig. 4.

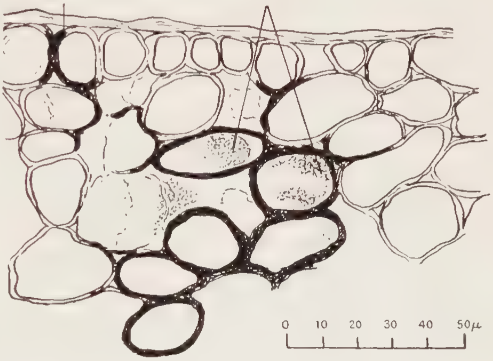
Necrotic suberin-containing cells almost at the surface of the cotyledon
Granular deposits staining yellow with potash
0 10 20 30 40 50μ

Fig. 5.

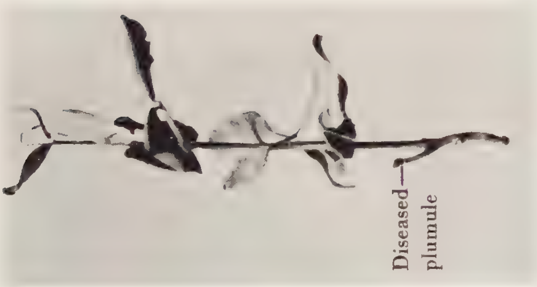
Diseased plumule

Fig. 2.

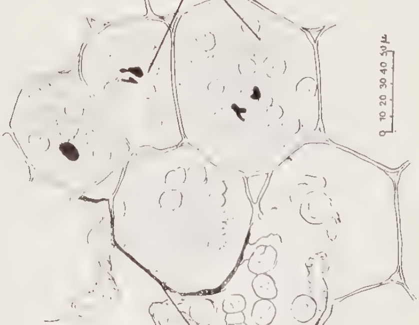
Healthy cells
Thickened walls of diseased cells
0 10 20 30 40 50μ

Fig. 3.

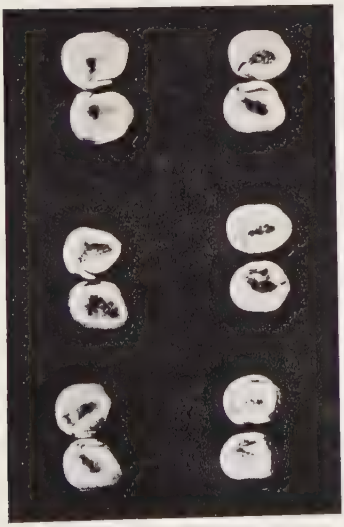

Fig. 1.

LACEY.—Studies in Bacteriosis (pp. 621–640).

PSYLLIA PEREGRINA FÖRST., THE HAWTHORN RACE OF THE APPLE SUCKER, *P. MALI* SCHMIDB.

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In the psyllid population of hawthorn trees of south-east Scotland, *Psyllia mali* Schmidb., an insect which has long been known to breed only on species of *Pyrus*, was found to predominate. *Psyllia melanoneura* Först. was present in some localities in equally large numbers and also a few specimens of the so-called species, *P. peregrina* Först. It was soon discovered, however, that the various stages of *mali* and the earlier occurring "*peregrina*" of hawthorn were practically indistinguishable. The two were found to differ merely as regards size and coloration, but the differences were neither large nor constant. Further, the adults of the hawthorn-reared forms of the *mali-peregrina* complex were morphologically indistinguishable from adults of *P. mali* reared on apple, but differed in their immature stages from those of the latter. The problem that thus arose was two-fold: first, to ascertain whether the forms reared on hawthorn and variously identified as *P. peregrina* and *P. mali* were members of one and the same species; and second, to discover their exact relationship with *P. mali* of apple. With these aims in view the following procedures were adopted:

(1) Rearing adults from batches of eggs laid on hawthorn twigs by females at different times during the preceding autumn, in the field as well as in the greenhouse, and identifying the insects which emerged.

(2) Comparison of adult *mali* and "*peregrina*" from hawthorn with each other and with *mali* from apple.

(3) Comparison of the nymphs of the hawthorn forms with nymphs of *mali* from apple.

(4) Experiments designed to test the survival capacity of the hawthorn-reared nymphs when transferred to apple and *vice versa*.

(5) Mating experiments with adults bred respectively on hawthorn and apple, and observations as to differential oviposition on hawthorn and apple.

(6) Comparison of the life histories of the forms on hawthorn and on apple.

LIFE HISTORY.

The life history of the two psyllids on hawthorn and apple is similar and does not differ materially from that of the apple sucker in England, described by Awati (1915). In south-east Scotland the adults of both appear towards the end of May and may be found in the field till the end of October. Both insects die after laying their eggs on the twigs of their hosts in September and October, and the winter is passed in the egg stage. Hatching occurs in the first week of April, and the nymphs, especially the first three instars, are gregarious, concealing themselves between the folds of the leaf buds. The nymphs, as is the rule in most Psyllidae, moult five times, and wing pads appear after the first ecdysis in the apple forms, and a little later, sometimes not before the second ecdysis, in the hawthorn forms. There is no difference between the habits of the nymphs of the two forms.

Pyrus malus is the usual host plant of the apple form and *Crataegus oxycantha* of the hawthorn form, but other species of the two genera are also attacked with varying degrees of intensity, and, on the other hand, there are certain species which are almost entirely neglected. An idea of the degree of preference shown was obtained by examining the twigs of various hosts for eggs at the height of the egg-laying season. The results are shown in Table I.

Table I.

Species of *Pyrus*	Eggs laid	Species of *Crataegus*	Eggs laid
1. *malus*	Very plentiful	1. *oxycantha*	Very plentiful
2. *prunifolia* var. *colvellei*	,,	2. *blanchardi*	,,
3. *baccata* var. *purpurea*	,,	3. *tennowana*	,,
4. *aucuparia*	Plentiful	4. *pasgens*	,,
5. *prunifolia* var. *pendula*	,,	5. *populnea*	,,
6. *elevi*	,,	6. *sorbifolia*	,,
7. *communis*	,,	7. *heldreichii*	,,
8. *salicifolia*	Scarce	8. *pyrifolia*	Plentiful
9. *oblonga*	,,	9. *crus-galli*	,,
10. *commixta*	Very scarce	10. *hupchensis*	Scarce
11. *amygdaliformis*	,,	11. *mollis*	,,
12. *pubescens*	,,	12. *pinnietifida*	,,
13. *canescens*	,,	13. *nitida*	,,
14. *pinnatifida*	,,	14. *coccinea*	,,
		15. *penitagyna*	Very scarce

These observations were made in the Royal Botanic Gardens, Edinburgh, where trees of both genera were growing on the same lawns and under exactly similar conditions.

MORPHOLOGY OF ADULTS.

The forms of "*peregrina*" emerging in spring are green insects, noticeably smaller than *mali* of the apple. A large proportion of the nymphs, however, complete their life cycle later in spring and in early summer, and from these emerge adults which closely approach the apple sucker in size and coloration and were invariably identified as such.

The chief characters used to separate species of Psyllidae are the shape and size of antennae, genal cones and the vertex, and the shape, size, and colour of the elytra and their veins, and of the genitalia. In all these respects the two forms from hawthorn and apple resemble each other. Both have the antennae about twice the length of the head width, with the last two segments invariably, and apices of segments 4–8 often, dark. Genal cones are somewhat pubescent and roughly triangular, their length less than the vertex down the middle and their divergence not more than the base of either. Both insects have typically the same shape and size of elytra with relatively long prostigma and pale yellowish to brownish veins. The male genitalia of both are pubescent and have the anal valves moderately long and broad with bluntly rounded apices. The forceps are narrower, slightly shorter, ending in black claws and equally pubescent. The female genitalia of both have the anal valve slightly longer than the subgenital, abruptly narrowed to a blunt point in the distal third and pubescent.

The only difference noticeable in the two insects is with respect to their size, the hawthorn forms being slightly smaller, especially in early summer, than those from the apple and also less stout. The former measure 2–3 mm. in length, the latter 3–3·5 mm. This difference, however, is inconstant, and occasionally adults of the hawthorn form are encountered which are stouter than the average and of the apple form smaller than the average.

MORPHOLOGY OF NYMPHS.

In general shape and size nymphs of the hawthorn form resemble those of the apple sucker. Both measure 2–2·5 mm. in length. The most characteristic difference, however, is the presence of a longitudinal brown streak on the wings of the last instar of the former which is absent in apple-sucker nymphs. These streaks are not apparent at all in a small proportion of the nymphs. Another difference occurs in the setae surrounding the abdominal margin of the two nymphs. In the hawthorn forms, they are all of one kind, long and spatulate; in the apple sucker they are of two kinds, long, pointed, variously curved setae and smaller, spear-shaped setae. The nymphs of the former have also round tubercles on the dorsum in place of the very minute setae that invest the body of the latter. The circumanal pores are nearly similar in both.

EXPERIMENTS ON OVIPOSITION.

Adults from hawthorn and apple were collected in the field from the end of August to the beginning of October, and were confined on each other's host plants in small cellophane cages enclosing small twigs and having their entrances closed by means of cotton-wool. The experiments were repeated many times and the results were always negative, no eggs being laid. Control experiments were also made in which insects were put on their own food plants and eggs were laid, showing that the conditions prevailing in the greenhouse did not preclude oviposition. Details are given in Table II.

Table II.

Original host	Number of specimens	Transferred to	Number of eggs laid	Duration of experiment
Hawthorn	30 males and females	Apple	0	Sept. 1–12
„	20 „	„	0	Sept. 20–24
„	20 „	„	0	Sept. 20–Oct. 1
„	20 „	„	0	Oct. 7–14
„	15 „	Hawthorn	12	Sept. 24–Oct. 14
„	16 „	Pear	2	Oct. 7–14
Apple	25 „	„	10	Sept. 24–29
„	25 „	„	26	Sept. 29–Oct. 7
„	25 „	Mountain ash	4	Sept. 24–29
„	40 „	„	7	Sept. 29–Oct. 7
„	25 „	Hawthorn	0	Sept. 7–24
„	20 „	„	0	„
„	25 „	„	0	„

EXPERIMENTS ON MATING.

These were performed by confining large numbers of adults, from apple and hawthorn, in large glass jars supplied with twigs of both trees stuck in moist sand. The top of the jar was covered with muslin and care was taken that insects of one sex from one host were confined with insects of the opposite sex from the other host. In this way it was ensured that males and females copulating inside the glass jars were derived from different hosts. This experiment was repeated many times and was also performed by confining the adults in cellophane cages on twigs of plants in pots. No case of mating was observed, although there were many successful pairings in control jars wherein adults reared on the same host were confined.

TRANSFERENCE OF NYMPHS TO DIFFERENT HOSTS.

To test their survival capacity when put on different food plants, nymphs, of all instars, from apple and hawthorn were put on hosts other than their own. This was effected either by confining the nymphs on fresh leaves in small wide-mouthed jars or putting them on labelled, growing twigs of a potted plant. The nymphs travel little, and so the possibility of their migrating to neighbouring twigs is remote. To obviate such a contingency, however, a band of cotton-wool, which the nymphs could not cross, was attached round the bases of the twigs. The results are given in Table III.

Table III.

Original host	Number of nymphs	Host to which transferred	Duration of experiment	Result
Apple	18 I, 2 II*	Hawthorn	April 7–17	The majority of the nymphs remained alive during the whole period, secreting and moulting
,,	15 III and IV	,,	April 17–May 5	Do.
,,	18 I and II	Raspberry	April 7–8	All died in a short time
,,	20 I and II	Mountain ash	April 7–17	A few remained alive and secreted during the whole period
,,	20 I	Pear	April 7 onwards	Many were reared to adult stage
Hawthorn	20 I and II	Apple	April 7–11	A few remained alive and secreted during the whole period. None alive after April 11
,,	7 II and III	,,	April 17–25	A few remained alive and secreted during the whole period. None alive after April 25
,,	20 IV	,,	April 26–May 2	Only one nymph survived this period

* The Roman numerals indicate the instars of the nymphs.

DISCUSSION.

Since 1848 when it was first described by Förster, *Psyllia peregrina* has been treated by most authors as a species distinct from *P. mali*. Scott (6) in describing the nymph and adult doubtfully gave *P. mali* as one of its synonyms. *P. peregrina* and *P. mali* were also recognised as two separate species by Edwards (3) and by Aulmann (1). Sulc, however (*vide* his synonymy of *P. mali*, cited by Speyer (7)), regarded *P. peregrina* as a synonym of *P. mali*. In the descriptions of the former given by Löw (5), Scott (6), Edwards (3) and Kuwayama (4) there is little that is not equally applicable to *P. mali*, and the same may be said of the descriptions of the latter given by various authors from time to time, with respect to *P. peregrina*. Löw's (*loc. cit.*) and much of Scott's (*loc. cit.*) descriptions of *P. peregrina* are based on the colour of the various parts of the body, a very inconstant feature in the genus *Psyllia*. Edwards (*loc. cit.*) separates *P. mali* from *P. peregrina* because of their different food plants and the difference in their size. The latter is not a reliable criterion, and late in summer and in early autumn, insects reared on hawthorn are about as robust as the typical apple sucker.

The two insects from apple and hawthorn are thus morphologically indistinguishable as adults, but, as stated before, differ in their nymphal stages. The question of their correct systematic position, therefore, largely turns upon what we accept as the criteria of specific separation in these insects. It seems to the present writer that in Psyllidae at least, differences of biology, host plants and even of immature stages, can serve only as confirmatory evidence in the differentiation of species, the identity of which must be primarily based on fixed peculiarities of adult morphology. Judged by this test, *P. peregrina* is not specifically distinct from *P. mali* and the two must be regarded as one species.

Experiments detailed in the foregoing pages have shown that the insects on hawthorn and on apple are closely confined to their individual food plants, and the nymphs of the one do not live long when transferred to the host plant of the other. Nor do they show any tendency to mate with each other or lay eggs on each other's host plants. In view of these facts it is concluded that *P. mali* on apple has a race on hawthorn inseparable from it in adult morphology but with differences in biology and immature stages. To this latter is given the name of *P. mali* Schmidberger, race *peregrina*.

As a rule the members of two biological races of a single species are morphologically identical in all their post-embryonic development. There are exceptions, however, of which two instances have been cited by Thorpe (9), one being *Paraphorocera senilis*, a Tachinid parasite of the European corn-borer investigated by Thompson (8), and the other *Pales pavida* Meig., also a Tachinid; in both of these the biological races were indistinguishable as adults but showed constant differences in larval morphology, a phenomenon to which the term *poecilogony* has been applied. As pointed out by Thorpe (*loc. cit.*), such cases are rare among insects, but that they can exist has been mathematically shown by Thompson (*loc. cit.*), who also asserts that poecilogony cannot be considered to furnish any evidence either for convergence or divergence in evolution, because it can be understood without reference to either. This may be true theoretically, but a static existence with no evolutionary change of any kind seems to be the least likely thing in nature, and two closely allied forms will probably grow either more or less alike in course of time. It has been pointed out that a small proportion of the nymphs of the hawthorn form of *P. mali* do not show the brown longitudinal streak on their wing pads, a characteristic which, more than any other, distinguishes them from the nymphs of the apple form. This might be taken to show that in cases of poecilogony, while the characters separating the larvae of two biological races are fairly constant, yet, in a small number of individuals, some of the characters of one may approximate to those of the other and hence lead to greater similarity between the two. This may not, however, necessarily mean an obliteration of the differences in their biology.

It is at best only possible to speculate whether such cases will lead to a convergence to one species or to a divergence into two. Thorpe (*loc. cit.*) is of opinion that they can be explained more satisfactorily on the hypothesis of evolutionary divergence than of convergence. This explanation might possibly apply also to the races of *P. mali*, where the nymphs remain in intimate contact with their hosts throughout life, and any changes of structure that might be attributable to differences of food plant would be more likely to appear in the nymphs than in the adults.

CONCLUSIONS AND SUMMARY.

The conclusions based on these investigations may be summarised as follows:

1. "*P. peregrina*" and *P. mali* of hawthorn are identical, and are only the seasonal forms of one and the same species. These insects are also morphologically indistinguishable from *P. mali* on apple.

2. The nymph of the forms occurring on hawthorn is different from the typical nymph of the apple sucker (*P. mali*), and has long been known as the nymph of "*P. peregrina*." In it, however, there occurs a small percentage of variations, in which the characteristic brown longitudinal streak of the wing pads does not occur.

3. Although morphologically similar, specimens of *P. mali* of hawthorn and apple do not mate with each other, nor do they oviposit interchangeably on each other's host plants. The nymph of either when transferred to the food plants of the other does not survive very long.

4. *P. mali* of the apple has a biological race on hawthorn, which has long been known as *P. peregrina*. This race should be distinguished as

P. mali Schmidb. race *peregrina*, which is the name it is proposed to assign to it. The two races, in view of their dissimilar nymphs, furnish an instance of poecilogony.

I wish to express my grateful thanks to Dr A. E. Cameron for advice given during the progress of the work and for much helpful criticism in the preparation of the manuscript. I am also indebted to Mr F. Laing for confirming or correcting the identification of the Psyllids submitted to him, and to the Trustees of the Moray Endowment Fund of Edinburgh University for a grant, which helped to defray part of the expenses of this work.

NOTE. In a letter to *Nature*, Dec. 16, 1933, I used the racial name *crataegi*. In view of the fact that the insect was previously known as *P. peregrina*, I have decided on further consideration to adopt the old specific name *peregrina* as that of the race, in preference to *crataegi*.

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THE INSECTICIDAL PROPERTIES OF SOME EAST AFRICAN PLANTS. I.

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Introduction.

One item in the programme of the East African Agricultural Research Station, at Amani, is the examination of local plants with a view to finding (1) new insecticides which may be generally useful, and (2) insecticides, which, although not necessarily new, may be obtained from local wild or cultivated plants with sufficient ease and cheapness to make them more economical in use than imported poisons. This work has, therefore, naturally been divided into two parts: firstly, an examination of a number of plants, mostly reputed to be poisonous, for any insecticidal properties; and secondly, a more thorough investigation into those plants which show promise of real value as insecticides.

There are many plants in East Africa which possess either real or reputed poisonous properties. Many of those examined during the course of this investigation are stated, by the natives, to be of use as fish poisons: several of them were suggested to the author by Mr Greenway, systematic botanist at Amani.

In recent years the cultivation of plants of known insecticidal value from other parts of the world, such as pyrethrum and derris, has been undertaken by planters in various parts of East Africa. The pyrethrum grown in Kenya is undoubtedly of high toxic value, more so than any other plants examined, but it is not a particularly easy crop to handle and will only flower in certain districts of sufficient altitude and correct climate: it does not flower in Amani. In addition, the dried pyrethrum powder loses its toxic properties rapidly in the hot and damp East African climates. Derris grows more readily and over wider areas than pyrethrum, but the results obtained from the plants growing in Amani have been somewhat disappointing.

It is evident, therefore, that there is room for an effective insecticidal plant which is of easy and widespread cultivation, even if not quite as efficient as pyrethrum. *Tephrosia vogelii* appears to meet these requirements, and accordingly a considerable amount of work has been carried out on this plant.

One of the chief difficulties encountered has been the lack of suitable insects on which to test the plant extracts. Amani is singularly free from the usual insect pests, and we have not at present any suitable means of breeding them in large quantities. A fair number of *Citrus* aphides (probably *Toxoptera aurantii* Boy.) is usually available, and these together with caterpillars, which are moderately plentiful, have been used to test all the different extracts. The lack of insects has, however, limited the number of plants which could be tested, only the more hopeful ones being tried.

METHODS OF PREPARING AND TESTING THE EXTRACTS.

The method of extracting the plant materials has been standardised so that comparative results can be obtained with different plants, and, unless the contrary is stated, this standard method has always been employed. The plant material was dried either at 100° C. in an air oven or at 50° C. in a vacuum oven, when loss of any of the poisonous principle was suspected at the higher temperature. The dried material was ground as finely as possible in a high-speed laboratory mill and at once added to absolute alcohol in the cold. For every gram of material 9 c.c. of alcohol were used. The mixture was shaken vigorously and left for 24 hours with occasional shaking, after which it was filtered on a Buchner funnel and washed through with sufficient alcohol to bring the volume up to 10 c.c. per gram of material. This solution was now ready for use and is, throughout this paper, called a 10 per cent. extract of the plant material. If it is diluted to ten times its volume it becomes a 1 per cent. extract, and similarly for other dilutions.

When other methods of extraction have been used, they are described under the plant in question.

In order to obtain comparative results with different extracts it is essential to standardise carefully the method of applying the solutions to the insects. After trying various methods an apparatus was found which is both simple to fit up and use, and is also capable of supplying the same amount of insecticide per unit area every time.

The apparatus used is an ordinary hand spray as supplied by cellulose paint makers for amateur home painting, and consists of a glass vessel, about 400 c.c. capacity, with a screw-on metal top carrying a horizontal nozzle and a press button for starting and stopping the spray. It is usually connected to a hand pump, but in order to ensure constant and uniform pressure a cylinder of compressed air, fitted with a regulating valve, was used in these experiments. If the pressure be set always to the same value (30 lb. to the square inch was used), and the nozzle be placed at the same distance from a vertical surface, then the same weight of spray per unit area can always be obtained by pressing the button down for the same number of seconds. If the time be increased, the weight of spray is directly proportional to it.

It is not necessary to place the solution in the reservoir of the sprayer; it may be placed in a small tube into which the suction tube of the sprayer fits, thus allowing a small quantity to be used. By this method it is also possible to deliver a definite volume of spray, care being taken to prevent loss in the suction tube and nozzle by first spraying out a small amount so as to wet these parts, and then placing the measured volume of spray in the tube.

The degree of accuracy of this spraying device is shown by the following figures, obtained by placing successive filter papers at the same distance from the nozzle and weighing them before and after spraying. Filter papers of a diameter of 5·5 cm. were used, and the diameter of the area of wetting by the spray being about 30 cm. the filter papers received a quite even wetting if placed in the centre of the field of the spray.

As a result of preliminary tests the most suitable and convenient method of spraying was found to be for 4 sec. duration from a distance of 18 in., and this was always followed unless otherwise indicated.

At a distance of 18 in. filter papers sprayed for 4 sec. showed increases in weight of 0·367, 0·371, 0·372, 0·363, 0·368 and 0·370 gm., *i.e.* a mean of 0·370 gm. per paper, or 0·0156 gm. per sq. cm.: sprayed for 2 sec. at 18 in. increases of 0·180, 0·187, 0·191, 0·177, 0·185 and 0·186 gm. were found, *i.e.* a mean of 0·184 gm., or 0·0077 gm. per sq. cm.

Most of the tests with plant extracts were for 4 sec. spraying, so that for an aphis, which has an approximate area of 0·02 sq. cm., the amount of spray which it receives (assuming that all the spray that hits it stays on, which is unlikely) is 0·000312 gm. In the case of tephrosin sprays the minimum strength required to produce 100 per cent. kill is 0·002 per cent., so that each insect receives at the most 0·00000062 gm. of tephrosin: this indicates the extraordinary killing power of these compounds.

When testing the sprays against aphides a whole leaf was taken from the tree and the insects picked off until the required number remained on the leaf (on one side only). In this way any risk of injury to the test insects was avoided. The leaf was then pinned in a vertical position and sprayed for the required time with the sprayer nozzle horizontal (*i.e.* at right angles to the leaf). Excess of liquid was allowed to drain off and the leaf then placed in a vertical position in a glass dish and put on one side for observation.

Caterpillars were placed on a filter paper in a shallow glass dish which was inclined at an angle of about 45°, and the sprayer nozzle held at right angles to it. The caterpillars after spraying were placed on a dry filter paper and put aside in a glass dish with some leaves of their food plant.

As a standard of comparison for the various plant extracts, nicotine sulphate has been used throughout. Solutions were made up from the commercial product "Black Leaf 40," the nicotine content being taken as 40 per cent.

Both 1 per cent. saponin and ½ per cent. soft soap solutions have been used as spreaders, the simplest method being to make up a quantity of the spreader and use it instead of water for diluting the concentrated plant extract.

Controls, untreated and sprayed with spreader only, were included in every test.

The method of assessing the effect of the sprays has been to take ten aphides for each trial and to examine them 24 hours after spraying. For each insect unaffected no marks are given, for each slightly affected but likely to recover 2½ marks are given, for each badly affected and likely to die 5 marks, and 10 marks for every insect dead or moribund. These four groups are given in all the tables under the headings A, B, C and D. The total points thus awarded are given under the heading "%," 100 points being the maximum possible, for 10 deaths.

In the later trials a larger number of insects were used and the percentage kill only was calculated.

INSECTICIDAL PROPERTIES OF VARIOUS PLANTS.

Table I shows the results for seven different plants, using 1 per cent. saponin as spreader. Two trials were made except when otherwise indicated in the right-hand column. It will be seen that pyrethrum and *Tephrosia vogelii* are the only plants among these which can compare with nicotine in toxicity.

Table I.

Toxicity of various plant extracts to Citrus *aphis.*

Material used	Part of plant	% extract	A	B	C	D	%	No. of trials
Control—nil	—	—	10	—	—	—	—	4
1 % saponin solution	—	—	9	—	—	1	10	4
0·5 % nicotine as sulphate	—	—	—	—	—	10	100	—
0·25 % ,, ,, ,,	—	—	—	—	1	9	95	—
Pyrethrum	—	0·5	—	—	—	10	100	3
	—	0·25	—	—	—	10	100	3
Tephrosia vogelii	Seeds	0·5	—	—	—	10	100	—
		0·25	—	1	3	6	77½	3
	Leaves	1·0	—	—	1	9	95	—
		0·5	—	2	1	7	80	—
	Pods	1·0	—	1	3	6	77½	3
		0·5	2	3	4	1	37½	—
Cassia didymobotrya	Leaves	2·0	—	—	2	8	90	3
		1·0	—	3	5	2	52½	—
	Seeds	2·0	1	—	3	6	75	—
		1·0	2	2	6	—	35	—
Dichapetalum ruhlandii	Leaves	2·0	7	2	1	—	10	—
		1·0	7	3	—	—	7½	1
Lonchocarpus sp. A	Leaves	2·0	2	2	4	2	45	—
		1·0	5	2	2	1	25	—
	Roots	2·0	3	—	4	3	50	—
		1·0	3	3	2	2	37½	—
Lonchocarpus sp. B	Leaves	2·0	4	1	3	2	37½	—
		1·0	6	—	4	—	20	—
	Roots	2·0	4	1	2	3	42½	—
		1·0	5	—	3	2	35	—
Barringtonia racemosa	Bark	2·0	—	—	1	9	95	4
		1·0	—	2	—	8	85	4
		0·5	1	2	3	4	60	—

In Table II are given the results for thirteen more plants, using ½ per cent. soap as spreader. In both trials the spray was applied for 4 sec. from a distance of 18 in.

Pyrethrum (*Chrysanthemum cinerariaefolium* Trev.).

This plant is cultivated to some extent in the Kenya Highlands. The sample tested during this work was from a supply of freshly gathered flowers in Kenya and reached Amani not more than 2 weeks old.

The alcoholic extract is highly toxic to *Citrus* aphis, as shown in Tables I and II.

Table II.

Toxicity of various plants to Citrus *aphis.*

Material used	Part of plant	% extract	A	B	C	D	%	No. of trials
½ % soft soap	—	—	9	—	1	—	5	12
Control—nil	—	—	10	—	—	—	—	8
0·5 % nicotine as sulphate	—	—	—	—	—	10	100	8
0·25 % ” ” ”	—	—	—	—	2	8	90	3
Pyrethrum	—	0·25	—	—	1	9	95	4
	—	0·125	—	2	4	4	65	—
Tephrosia vogelii	Seeds	0·5	—	—	—	10	100	4
		0·25	—	2	1	7	80	—
	Roots	2·0	5	2	3	—	20	—
		1·0	8	1	1	—	7½	1
T. toxicaria	Leaves	2·0	—	1	2	7	82½	3
		1·0	—	1	3	6	77½	—
		0·5	3	3	—	4	47½	—
	Seeds	2·0	—	—	4	6	80	3
		1·0	2	1	4	3	52½	—
	Roots	1·0	—	—	—	10	100	—
		0·5	—	—	1	9	95	4
		0·25	—	1	1	8	87½	4
		0·125	4	2	3	1	30	—
T. candida	Leaves	2·0	2	4	2	2	40	—
		1·0	5	1	4	—	22½	1
	Seeds	2·0	3	1	5	1	37½	—
		1·0	4	4	1	1	25	1
	Roots	2·0	—	1	3	6	77½	3
		1·0	1	2	4	3	55	3
T. noctiflora	Leaves	2·0	8	2	—	—	5	—
	Seeds	2·0	8	1	1	—	7½	—
Tephrosia sp., probably *T. nyikensis* Bak.	Leaves	2·0	—	2	3	5	70	4
		1·0	1	4	1	4	57½	—
	Seeds and pods	2·0	2	4	—	4	50	—
Acokanthera longiflora	Leaves	2·0	9	—	1	—	5	—
	Stems	2·0	7	2	1	—	10	—
Cassia didymobotrya	Leaves	2·0	—	1	—	9	92½	3
		2·5	—	—	—	10	100	3
	Seeds	2·0	—	2	6	2	55	—
C. multijuga	Leaves	2·0	10	—	—	—	0	—
	Seeds	2·0	9	1	—	—	2½	—
C. bacillaris	Leaves	2·0	7	1	2	—	12½	—
	Seeds	2·0	7	2	1	—	10	—
C. laevigata	Leaves	2·0	6	4	—	—	10	—
	Seeds	2·0	7	2	1	—	10	—
Calophyllum inophyllum	Bark	2·0	—	6	4	—	35	—
		1·0	6	3	1	—	12½	1
Paullinia pinnata	Leaves	2·0	3	1	3	3	47½	—
		1·0	5	3	2	—	17½	1
Euphorbia tirucalli	Stems	2·0	—	1	4	5	72½	—
		1·0	1	5	2	2	42½	—
Derris elliptica	Root	2·0	—	—	—	10	100	—
		1·0	—	—	2	8	90	—
		0·5	—	4	2	4	60	—
		0·25	5	2	3	—	20	—
Hydnocarpus wightiana	Leaves	2·0	2	2	1	5	60	—
		1·0	1	1	6	2	52½	—
	Bark	2·0	4	2	3	1	30	—

In Table III are given results of some further tests with this sample. As before, the sprayer was held 18 in. off and the spray applied for 4 sec. They show that the fresh material is highly toxic and that if stored in a closed bottle the toxicity is not diminished over 3 months. If, however, it is kept in the open for 3 months its toxicity is very greatly reduced, and even 1 week under these conditions reduces its killing power by 10–15 per cent. The alcoholic extract kept in the open over 3 months loses toxicity only to a slight extent.

The use of pyrethrum in paraffin sprays is discussed later.

Table III.

Toxicity of various pyrethrum extracts to Citrus *aphis.*

Extract used	% extract	A	B	C	D	%
Alcoholic, on fresh sample	2·0	—	—	—	10	100
	1·0	—	—	—	10	100
	0·5	—	—	—	10	100
	0·25	—	—	—	10	100
	0·125	—	1	3	6	77½
	0·0625	2	5	2	1	32½
Alcoholic, on sample after storing for 3 months in closed bottle	1·0	—	—	—	10	100
	0·5	—	—	—	10	100
	0·25	—	—	1	9	95
	0·125	—	—	4	6	80
Alcoholic, on sample left in open for 3 months	1·0	—	4	4	2	50
	0·5	5	3	2	—	17½
Alcoholic, on sample left in open for 1 week	1·0	—	—	2	8	90
	0·5	—	1	2	7	82½
	0·25	—	2	1	7	80
Alcoholic extract (of fresh sample) left in open for 3 months	1·0	—	—	—	10	100
	0·5	—	—	1	9	95
	0·25	—	—	2	8	90

Genus Tephrosia.

(1) *T. vogelii* Hook. This plant is very widely spread throughout East Africa, growing wild in many parts and also being easy to cultivate. It is of considerable use as a cover crop and for contour hedges, and thus could be made to serve a double purpose if also used for the production of insecticides.

A considerable amount of work has been done on this plant and it is discussed in detail later in the paper (see p. 659).

(2) *T. toxicaria*. This plant does not appear to be indigenous to East Africa, but it grows readily in Amani, and is used as a fish poison in other countries. The roots are the most toxic part of the plant, a 1 per cent. extract giving 100 per cent. kill, a 0·5 per cent. extract 95 per cent. kill. The leaves and seeds although appreciably toxic do not compare with either its own root or the leaves and seeds of *T. vogelii*. These results are in agreement with those obtained by Tattersfield, Gimingham and Morris (6) for *T. toxicaria* from British Guiana. It is not likely to compete

with *T. vogelii*, as the plant is much less prolific and it is far easier to harvest leaves and seeds than to collect roots.

(3) *T. candida.* This again does not appear to be indigenous to East Africa but grows fairly well in Amani. The roots are moderately toxic, a 2 per cent. extract giving 77½ per cent. kill: the leaves and seeds are not appreciably toxic. These results are also in agreement with those of the above workers for *T. candida* from the West Indies.

(4) *T. noctiflora.* A small growing shrub; it has no insecticidal value whatever.

(5) *Tephrosia* sp., probably *T. nyikensis* Bak. An erect shrub; 8 ft. high; leaves and pods similar in appearance to *T. noctiflora.* It is said to be used by the natives around Mufindi, Iringa Province, as a fish poison and to be more toxic than *T. vogelii.* Only a small amount of dried leaves and pods was available and the trials indicated a toxicity much below that of *T. vogelii.* It is hoped to obtain larger quantities of fresher material for further trials, but loss of toxicity on drying is unlikely, as tephrosin and allied substances are very stable.

Genus Cassia.

(1) *C. didymobotrya* Fres. This is a fairly widespread shrub, indigenous to East Africa. It is said to be used by the natives as a fish poison. The seeds are only moderately toxic to aphis but the leaves possess a fair degree of toxicity, a 2 per cent. extract giving a kill of 90–92½ per cent., and a 2½ per cent. extract a kill of 100 per cent. The leaves are thus slightly less than half as toxic as *Tephrosia vogelii* leaves, and although it would not be advantageous to plant out *Cassia didymobotrya* in place of *Tephrosia vogelii*, it might be more economical to use it where it is growing wild (using the spray 2–2½ times more concentrated) than to cultivate *Tephrosia.*

So far no crystalline compounds have been obtained from the extracts. On evaporating the alcoholic extract of the leaves to dryness a sticky mass is obtained which is mostly soluble in ether; the residue, which is a pale yellow amorphous solid, amounting to 0·14 per cent. of the leaf, has so far failed to yield a crystalline product from the ordinary solvents.

The ether-soluble portion on evaporation yields 2·21 per cent. of an oily resinous mass, and as this was only slightly toxic it was not separated further.

The two substances were dissolved in alcohol and tested for toxicity (see Table IV). The results show that the solid product is, weight for weight, about half as toxic as the original extract: the method of isolation has probably destroyed some of it. It is being investigated further. The leaves of this plant are evidently sufficiently toxic to warrant its trial as an insecticide on estates on which it grows wild.

Table IV.

Toxicity of Cassia didymobotrya *extracts to* Citrus *aphis.*

Material	% of leaves	A	B	C	D	%
Amorphous solid	0·05	—	—	—	10	100
	0·005	—	—	2	8	90
	0·0025	—	4	3	3	55
Oily resin	1·0	1	5	3	1	37½
	0·5	8	2	—	—	10

(2) *C. multijuga*, *C. bacillaris* and *C. laevigata* were all examined and found to have practically no toxic properties.

Genus Lonchocarpus *and allied species.*

(1) *Derris elliptica*. The root of this plant already finds considerable use in other parts of the world as an insecticide, the chief poisonous principle being rotenone. Compared with results obtained elsewhere the toxicity of Amani-grown derris appears to be low; the rotenone content is also low.

The first samples examined from 1-year-old plants showed a rotenone content of only 0·7 per cent., and a 1 per cent. extract gave only a 30 per cent. kill with *Citrus* aphis.

Eighteen months later the same set of plants showed a rotenone content of 1·5 per cent., and a 2 per cent. extract gave a 100 per cent. kill, a 1 per cent. extract a 90 per cent. kill, and a ½ per cent. extract only a 60 per cent. kill. These figures are below those obtained by Tattersfield and his co-workers for *Derris elliptica* roots, and the results so far also indicate a low rotenone content, due either to the variety cultivated or to the local climatic or soil conditions.

(2) *Lonchocarpus* sp. A., possibly *L. capassa*. This is a large shrub growing wild in the Shinyanga district. It was examined in the hope of finding an indigenous plant of this genus with toxic properties. Neither the leaves nor roots are appreciably toxic.

(3) *Lonchocarpus* sp. B. From the same district as A and differing in being a smaller shrub with considerably smaller leaves. Also not appreciably toxic.

Other plants.

(1) *Paullinia pinnata.* The leaves of this plant have been reported to contain tephrosin by Hanriot(3) and other workers. An extract of these was not found to be appreciably toxic to aphis, 2 per cent. strength only giving a 47 per cent. kill.

(2) *Calophyllum inophyllum*, the bark of which is stated to be used as a native fish poison, was found to have little toxic effect.

(3) *Euphorbia tirucalli.* All parts of this plant are stated to act as a fish poison. A 2 per cent. solution was found to give a 72½ per cent. kill with *Citrus* aphis, showing some insecticidal effect. As the plant is not very widespread no further work was carried out with it.

(4) *Hydnocarpus wightiana*, also claimed to be a fish poison, was not found to be appreciably toxic to aphis.

(5) *Acokanthera longiflora*, stated to be used by the local natives as an arrow poison, had no insecticidal action.

(6) *Dichapetalum ruhlandii*, a bush which is poisonous to cattle and goats, is non-toxic to insects.

(7) *Barringtonia racemosa.* A tree, the bark of which is reported to be of use as a fish poison. The preliminary results, given in Table II, for extracts of the bark show that it has a moderate degree of toxicity, warranting fuller examination. Table V shows the results of further trials on the extracts and on some solid products obtained from them. The alcoholic extract was evaporated to dryness and left a residue which was partly soluble in water, giving an amorphous solid, equivalent to 0·5 per cent. of the bark by weight, and a resinous substance insoluble in water but soluble in ether, equal to 0·05 per cent. of the bark.

Table V.

Toxicity to Citrus *aphis of* Barringtonia racemosa.

Material	% of bark	Insects used	Moribund and dead	% moribund and dead
Control	—	40	3	7·5
1 % saponin	—	40	4	10
Alcohol extract	2·5	40	40	100
	2·0	50	49	98
	1·0	50	28	56
Cold-water extract	2·0	40	19	47·5
Hot-water extract	2·5	50	48	96
	2·0	50	47	94
0·01 % solid preparation	2·0	40	8	20
0·1 %	20·0	40	8	20
0·001 % resin	2·0	40	24	60
0·002 %	4·0	40	39	97·5
0·5 % *Tephrosia vogelii* seed	—	40	40	100
0·5 % nicotine as sulphate	—	40	38	95

These results show that a 2–2·5 per cent. alcoholic extract of the leaves is equivalent to a 0·5 per cent. extract of *Tephrosia vogelii* seeds (which is equivalent to a 1 per cent. extract of *T. vogelii* leaves). Extracted by boiling with water a 2–2·5 per cent. extract is practically as good, but a cold-water extract is only half as efficient. Of the solid products obtained the resin contains the chief bulk of the toxic principles, but it requires a concentration equivalent to 4 per cent. of the bark to equal a 2 per cent. alcoholic extract. These products will be examined further.

INSECTICIDAL PROPERTIES OF *TEPHROSIA VOGELII* HOOK.

Of the numerous plants examined, *T. vogelii* has proved by far the most efficient as an insecticide, if ease of cultivation and storage are taken into account. Pyrethrum is slightly more toxic and is undoubtedly a quicker killer, but these considerations would only outweigh the advantages of *T. vogelii* in those areas where pyrethrum grows and flowers readily. Apart from the greater ease of cultivation, leaves and seed pods are far easier to collect than flower heads, which must be gathered at the right stage, as with pyrethrum.

The sample of *T. vogelii* examined by Tattersfield, Gimingham and Morris (5) was obtained from the Sudan and was described as having a violet flower (rarely white); text-books describe this plant in the same manner. In nearly all areas of East Africa it occurs with white flowers only: in a few areas, Lushoto being one, it occurs with bluish or violet flowers: there appears to be no botanical difference between the two varieties. Only the white-flowered variety has been examined so far, but it is proposed to compare the other with it when supplies can be obtained.

The toxicities of various parts of the plant are given in Table VI. All results are the means of several tests. It will be seen that the seeds have a high toxicity comparable to that of nicotine used as sulphate, while the leaves are somewhat more than half as effective. The stems and pods are much less toxic. In view of the work entailed in separating the seeds from the pods, an extract of the whole pods with seeds was tried; this had a toxicity of about one-quarter that of the seeds alone, and, in practice, if the seeds had to be removed by hand the use of the whole pods would probably be preferable.

The toxicity of the various parts is dependent on the amount of tephrosin present, and a series of determinations has been made to ascertain the tephrosin content in the different parts.

Table VI.

Toxicity of various parts of T. vogelii *to* Citrus *aphis.*

Part of plant	% extract	A	B	C	D	%
Leaves	2·0	—	—	—	10	100
	1·0	—	—	—	10	100
	0·5	—	1	2	7	82½
	0·25	—	5	4	1	42½
Stems	4·0	—	—	—	10	100
	2·0	—	1	4	5	72½
	1·0	4	2	3	1	30
Seeds	1·0	—	—	—	10	100
	0·5	—	—	—	10	100
	0·25	1	—	3	6	75
	0·125	4	3	2	1	27½
Pods, without seeds	4·0	—	—	—	10	100
	2·0	—	—	3	7	85
	1·0	—	2	3	5	70
Pods, with seeds	4·0	—	—	—	10	100
	2·0	—	—	—	10	100
	1·0	—	2	2	6	75
Roots	4·0	4	4	2	—	20
	1·0	8	1	1	—	7½
Flowers	4·0	8	2	—	—	5
Control, nil	—	9	1	—	—	2½
0·5 % soft soap	—	9	1	—	—	2½
0·5 % nicotine as sulphate	—	—	—	1	9	95
0·25 % ,, ,, ,,	—	—	—	3	7	85

The seeds, which contain the highest proportion of tephrosin, have received the most attention. The plant is a perennial and therefore it is more economical to use the seeds, which are produced in quantity, than to defoliate it. The seeds contain about 12 per cent. of a fixed oil and this somewhat complicates the extraction. After trying a number of methods the most suitable for either small or large quantities was found to be as follows: The crushed seed is exhausted in a Soxhlet type of extractor with 90 per cent. alcohol and the excess of alcohol is then distilled off until the amount left is equal in volume to the calculated volume of oil from the seed, based on a 12 per cent. content. When cold, an amount of water is added which is just insufficient to throw out the oil from solution (this involves diluting the alcohol to about 75 per cent. strength), a small quantity of ether is added when quantitative results are required, and after several hours' standing the whole mixture is filtered off on a Buchner funnel, the precipitated tephrosin being carefully washed with several small quantities of ether. If more ether be added to the filtrate a further small amount of tephrosin can be obtained and filtered off. The precipitate, which is yellow, is not pure tephrosin and must be recrystallised: it is referred to in this paper as "crude tephrosin."

After several trials the following was found to be the most rapid way of separating the crude tephrosin into its different components. It is dissolved in hot alcohol and allowed to crystallise out slowly, two sets of crystals being obtained, large white tabular ones and very small yellow needles. If the first crop be removed after 24 hours it consists largely of the yellow needles which are less soluble in alcohol than the white crystals: the second crop consists largely of the latter crystals. The crystals are washed in a little ether and then agitated in the cold with a small quantity of chloroform in which the yellow crystals are considerably the more soluble. In this manner they are separated and a further crystallisation from alcohol produces the two substances practically pure.

The yellow needles have a melting-point of 227° C. and the white crystals of 187° C. The proportion of yellow crystals in the crude tephrosin was approximately 20 per cent.

Table VII shows the amount of crude tephrosin and other products in various parts of the plant. In the leaves, stems and pods there is no appreciable amount of fixed oil, so that the alcoholic extract can be evaporated to small bulk and ether added to precipitate the tephrosin.

Table VII.

Percentage of "crude tephrosin" in different parts of T. vogelii.

Part of plant	Leaf	Stem	Pod	Seed	Root
% crude tephrosin	0·18	0·09	0·06	0·35	0·01
% tephrosal	0·008	Trace	0·05	0·01	Trace
% resin	0·02	Nil	0·10	0·08	Nil

In addition to these crystalline solids, resins were obtained from the leaf, pod and seed. These resins are soluble in ether and so may be easily separated from the crude tephrosin.

All parts of the plant contain tephrosal, an aldehyde of an oily nature: it is most readily obtained by steam distillation of the pods.

In Table VIII are shown the results of testing these various products on *Citrus* aphis. All the substances were dissolved in acetone and diluted with $\frac{1}{2}$ per cent. soft soap solution.

The crude tephrosin from the seeds is somewhat more toxic, weight for weight, than that from the leaves, and the white crystals more toxic than either. The yellow crystals on the other hand are practically non-toxic, as also are the three resins, the slight toxicity of the latter probably being due to the presence of a small amount of tephrosin. Tattersfield, however, found the resin that he isolated to be appreciably toxic whereas the various crystalline products were not: as he pointed out(6), the drastic method employed by him to separate them had probably affected their compositions.

Table VIII.

Toxicity of various products from T. vogelii *to* Citrus *aphis.*

Material	%	% of plant	Insects	Dead and moribund	% dead and moribund
Crude tephrosin from leaves	0·0036	2	10	10	100
	0·0018	1·0	40	39	97·5
	0·0012	0·67	20	18	90
Crude tephrosin from seeds	0·0036	1	10	10	100
	0·0024	0·67	20	20	100
	0·0012	0·33	30	30	100
	0·0006	0·17	40	35	87·5
White crystals (187° C.)	0·002	0·54	30	30	100
	0·001	0·27	30	30	100
	0·0005	0·14	30	29	97
	0·0002	0·05	30	14	47
Yellow crystals (227° C.) from seeds	0·06	100	40	6	15
	0·6	1000	40	15	37·5
Resin from seeds	0·08	100	40	15	37·5
Resin from leaves	0·02	100	40	11	27·5
Resin from pods	0·10	100	40	4	10
Tephrosal from pods	0·005	10	30	30	100
	0·0025	5	30	21	70
	0·00125	2·5	30	11	37

Tephrosal is an oily liquid with a strong, not unpleasant odour, its refractive index at 20° C. being 1·4912. It is an aldehyde combining with sodium bisulphite to give a white crystalline solid. It is somewhat toxic to *Citrus* aphis and, indeed, probably accounts for an appreciable proportion of the toxicity of the pods.

The white crystals of melting-point 187° C. are undoubtedly identical with those obtained by Hanriot. Clark (2) claims that they are a mixture of two substances, deguelin of melting-point 177° C. and tephrosin of melting-point 198° C. The author so far has been unable to separate them into components: five recrystallisations from acetone leaving the melting-point at 187° C. Clark states that the deguelin can be obtained from the mixture by destroying the tephrosin with chromic oxide in acetic acid: when the author tried this, about 80 per cent. of the crystals were destroyed, but the melting-point of those remaining was 186° C., showing practically no change.

The yellow crystals of melting-point 227° C. are probably dehydrodeguelin (melting-point 223–224° C.), and are possibly formed by the action of the boiling alcohol on tephrosin.

Specific uses of T. vogelii.

(1) As a contact insecticide against aphis, thrips (probably *Physothrips xanthocerus* Hood) and similar soft-bodied insects, extracts of *Tephrosia vogelii* are very effective and should eventually replace

entirely the use of nicotine in East Africa, either alone or in mixed sprays, against these insects. Nicotine sprays are comparatively expensive whereas *Tephrosia* sprays can be prepared very cheaply.

A number of tests with water extracts of the plant material has been made, using both cold and boiling water. The dried and ground material was either soaked in cold water for 24 hours or boiled with water for 30 min. (in some cases 60 min.), strained and diluted to the required strength, soft soap being added to make a ½ per cent. solution. Table IX shows the results obtained in toxicity tests.

Table IX.

Toxicity of extracts of T. vogelii *to* Citrus *aphis.*

Material	% extract	Insects	Moribund and dead	% moribund and dead
Alcoholic, leaf	1·0	40	39	97·5
	0·5	30	24	80
Alcoholic, seed	0·5	30	30	100
	0·25	30	25	83
Leaf, cold water	2·0	30	30	100
	1·5	30	29	97
	1·0	30	24	80
Leaf, boiling water for 30 min.	1·5	30	30	100
	1·0	30	29	97
	0·5	30	22	73
Do. for 60 min.	0·5	20	15	75
Seed, cold water	5·0	20	4	20
Seed, boiling water for 30 min.	2·0	30	30	100
	1·5	30	29	97
	1·0	40	38	95
Do. for 60 min.	1·0	40	37	92·5

The figures show that an aqueous extract of the leaf made with boiling water is as toxic as an alcoholic extract: made with cold water it is about 50 per cent. less toxic. With the seeds, boiling water gives an extract only half as toxic as an alcoholic extract, and cold water almost fails to extract any toxic principle: this difference is no doubt due to the presence of 12 per cent. of fixed oil in the seeds. Boiling for 60 min. is no more effective than for 30 min.

An extract of the leaves made with boiling water and using ½ per cent. soft soap as spreader has been used successfully against thrips on some experimental coffee trees in Amani.

Experience has shown that for home-made sprays on the farm the leaves are preferable to the seeds, although seed extracts are quite suitable if used somewhat stronger. The seeds, however, would be very suitable for large, or semi-large-scale extractions. As described earlier in the paper crude tephrosin is easily obtained, and if a suitable form in which to supply it to the public can be devised, it should find a ready sale in place of nicotine. Unfortunately it is a very insoluble substance, being almost completely insoluble in water and soluble to an extent of not more than 1–5 parts per 100 of the ordinary organic solvents. A large number of methods has been tried both with solvents and emulsions: of ordinary solvents pyridine appears to be the best, dissolving about 8 per cent. weight in volume. Emulsions have not proved very stable in concentrated form, but no efficient emulsifier is available in Amani: it is hoped to carry out further work on this point.

The most effective method found by the author for producing a prepared spray from the crude tephrosin is to dissolve it at the rate of 1 gm. in 5 c.c. of technical triethanolamine (trihydroxytriethylamine) heated to about 150° C.: it is readily soluble to that extent at this temperature. When dissolved 10 c.c. of oleic acid are added, forming the soap triethanolamine oleate, and the mixture is stirred into 437 gm. of ordinary soft soap, thus giving 1 gm. of tephrosin per pound of soap. Against aphis and thrips this has been found effective when dissolved at the rate of 1 lb. in 20 gallons of water. A sample of this soap after standing for 6 months was found not to have lost any efficiency when a portion taken from the top without stirring was tested. Diluted at the rate of 1 lb. to 20 gallons the mixture produces a spray containing ½ per cent. soft soap.

(2) *Paraffin sprays against* Antestia *and* Lygus *and for domestic uses.* A method of control against the bugs *Antestia* and *Lygus* on coffee trees which has found much favour in Kenya is the use of neat paraffin extracts of pyrethrum, as described by Le Pelley (4). A tent is placed over the tree and a quantity of the spray atomised inside it in a very fine mist, the idea being not so much to wet the insects with it as to fill the whole air with very fine particles of the spray. In a few minutes the insects fall to the ground dead or stunned and can be swept up.

Tephrosia vogelii seeds are readily extracted in the cold with paraffin by the same process as that described under "Methods of Extraction" for alcohol extracts.

These paraffin extracts have been tested in the laboratory against caterpillars, cockroaches and *Antestia*, together with similar extracts of some other plants, in comparison with pyrethrum solutions. The caterpillars and cockroaches were sprayed in open dishes and the *Antestia* in wire cages: the results are shown in Tables X and XI.

The figures show that weight for weight *Tephrosia* sprays are about half as toxic as pyrethrum under these conditions, and in addition they are slower acting. In Table XI are shown the results when using *Antestia*, the sprayer being held 2 ft. away and spraying for the times shown in column 2.

Table X.

Toxicity of some kerosene extracts to caterpillars (Nymphalidae *sp.*).

Material	Examined after	A	B	C	D	%
Kerosene	1 hour	8	—	1	1	15
	24 hours	8	1	—	1	12·5
1 % pyrethrum	1 hour	—	—	2	8	90
	24 hours	—	—	—	10	100
½ % pyrethrum	1 hour	—	—	4	6	80
	24 hours	—	3	1	6	72·5
2 % *Tephrosia vogelii* seed	1 hour	—	—	4	6	80
	24 hours	—	—	—	10	100
1 % *T. vogelii* seed	1 hour	—	7	—	3	47·5
	24 hours	—	5	2	3	52·5
0·006 % crude tephrosin	1 hour	—	—	5	5	75
	24 hours	—	—	—	10	100
0·003 % crude tephrosin	1 hour	—	4	3	3	55
	24 hours	4	—	2	4	50
4 % *Cassia didymobotrya*	1 hour	2	—	5	3	55
	24 hours	—	2	2	6	75
4 % *Barringtonia racemosa*	1 hour	2	1	4	3	52·5
	24 hours	2	—	3	5	65

Table XI.

Toxicity of paraffin sprays to Antestia.

Spray	Time	A	B	C	D	%
Paraffin	2 min.	8	1	1	—	7·5
	½ min.	9	1	—	—	2·5
Pyrethrum: 1 lb./gal.	½ min.	—	—	1	9	95
	¼ min.	—	1	—	9	92·5
	5 sec.	7	1	1	1	17·5
½ lb./gal.	½ min.	—	—	1	9	95
	¼ min.	5	3	2	—	17·5
Tephrosia vogelii seed: 2 lb./gal.	½ min.	—	—	1	9	95
	¼ min.	1	5	3	1	37·5
	5 sec.	9	1	—	—	2·5
1 lb./gal.	2 min.	4	2	2	2	35
	½ min.	6	1	2	1	22·5
Crude tephrosin: 2 gm./gal.	2 min.	2	—	2	6	70
	½ min.	3	5	—	2	32·5

It will be seen that paraffin extracts of *Tephrosia vogelii* seeds at the rate of 2 lb. per gallon are equal in effect to pyrethrum extracts of between ½ and 1 lb. per gallon. It must, however, be emphasised that in all these tests the insects were actually wetted by the spray; tephrosin will not kill insects unless it comes into actual wetting contact with them, and a paraffin extract of *T. vogelii* seeds, unlike a pyrethrum extract, is not effective in the form of a fine mist. This was demonstrated by Mr T. W. Kirkpatrick, who very kindly tested sprays for the author against

Antestia and *Lygus* in the field: he found the tephrosin-paraffin sprays useless when used under the same conditions as pyrethrum-paraffin sprays.

It is thus clear that *Tephrosia vogelii* cannot replace pyrethrum against these pests.

In the field of domestic sprays, paraffin extracts of pyrethrum have long been in use, and experiments have been made to ascertain if tephrosin sprays could replace them.

Some trials were carried out on cockroaches. Using 10 per cent. paraffin extracts of *Tephrosia vogelii* seeds and of pyrethrum, the former caused a 100 per cent. kill in 15 min. after spraying and the latter in 2 min. Using crude tephrosin in paraffin, a 1 in 1000 spray caused death in 1 min. and a 1 in 2000 spray in 5 min.

In further trials with cockroaches it was found that 10 per cent. pyrethrum sprays had a far quicker stunning effect than 10 per cent. *T. vogelii* seed sprays, but that the insects finally died (after several hours) sooner with the latter spray than with the former.

Against flies, mosquitoes and similar insects tephrosin has been found to be as effective as pyrethrum provided the insects are actually hit with it; pyrethrum, however, has a temporary repellent effect for a short time after spraying, a property which tephrosin does not possess; this could probably be remedied by adding some repellent such as oil of wintergreen (actually used in a well-known fly spray).

Tephrosin-paraffin sprays should therefore be able to replace pyrethrum extracts for domestic use for many purposes. The author has received very favourable reports from several people to whom he gave samples for testing. Further tests by interested persons are in progress.

(3) *Mosquito larvae control.* Occasional references (1) appear to the use of *Tephrosia* extracts as a poison against mosquito larvae. The author has carried out some tests on this interesting question. Sufficient anopheline mosquitoes were not available, so that the tests had to be made on the larvae of *Stegomyia* sp. Table XII shows the results obtained with different extracts using ten larvae in each test, and it is evident that tephrosin extracts are to some extent toxic to mosquito larvae, acting perhaps as stomach poisons since the larvae are surface breathers. The crude tephrosin preparation would be the most suitable when the water to be treated is required for domestic use of any kind, as the solution would be free from the other organic matter which would occur in direct extracts of the leaf. It should be noted that tephrosin is non-poisonous to human beings and animals.

Table XII.

Toxicity of Tephrosia *extracts to* Stegomyia *sp.*

Material	Dilution	Dead after 1 hour	After 24 hours
Crude tephrosin	1 in 100,000	8	10
	1 in 250,000	1	4
	1 in 500,000	Nil	Nil
Alcoholic extract of *Tephrosia vogelii* seeds	1 in 300	8	10
	1 in 500	2	4
	1 in 750	Nil	1
Alcoholic extract of *T. vogelii* leaves	1 in 100	9	10
	1 in 250	4	5
	1 in 500	Nil	Nil
Water extract of *T. vogelii* leaves	1 in 100	4	10
	1 in 250	Nil	5

(4) *Insect powders.* The finely-ground pod of *Tephrosia vogelii* has a certain value as an insect powder, due probably in the main to the tephrosal present in it: it cannot, however, compare with pyrethrum powders and probably would have no value in practice except perhaps as a filling in the latter.

The dried crude tephrosin also has a moderate toxic effect when dusted on to flies.

(5) *Fixed oil from seeds.* This oil, the nature of which has not yet been ascertained, yields on saponification a fatty acid of a red colour with an agreeable odour. On treatment with alkalies, this latter yields a soap which, while of no domestic use on account of the red colour which causes a slight staining, might be employed as a spreader for the sprays.

A few trials were made and it appeared to have a spreading power equal to that of ordinary soft soap.

RESULTS.

The results show that *Tephrosia vogelii* extracts are equal to, or even better than, nicotine for general spray purposes: they are somewhat less toxic and somewhat slower in action than pyrethrum sprays.

T. vogelii, however, possesses the very great advantage of being extremely widespread in East Africa, growing equally well in the hills up to between 5000 and 6000 ft. altitude (possibly higher), and on the plains down to sea-level.

SUMMARY.

1. A number of East African plants, mostly indigenous, has been tested for insecticidal value as contact poisons.

2. No new insecticide equal in toxicity to nicotine has been discovered.

3. Two plants, *Cassia didymobotrya* and *Barringtonia racemosa*, have toxic properties slightly less than half that of nicotine used as sulphate. The plants, which grow wild, should be of value for making insecticides on those farms where they are already growing: they would not, however, be worth cultivating. No definite chemical compounds were obtained from these two plants.

4. The toxicity of extracts of *Tephrosia vogelii* has been found to be of the same order as that of nicotine in the form of sulphate, and these extracts should be able to replace nicotine in all sprays against soft-bodied insects such as aphis and thrips.

5. Nearly all parts of this plant contain tephrosin, the seeds having the largest content at 0·35 per cent., whilst the leaves contain 0·18 per cent. Methods are given for the easy extraction of tephrosin from the various parts of the plant. The product obtained has been called "crude tephrosin" by the author, and in this form is suitable for the preparation of insecticides.

6. Crude tephrosin contains about 20 per cent. of a substance crystallising in fine yellow needles with a melting-point of 227° C., which is probably dehydrodeguelin and is non-toxic. The remaining 80 per cent. forms white tabular crystals with a melting-point of 187° C., and is undoubtedly identical with Hanriot's tephrosin. So far it has resisted separation into two or more constituents, either by repeated recrystallisation or by oxidation. This crystalline substance accounts for the toxicity of the crude tephrosin and of the plant itself.

7. Resins were obtained from most parts of the plant but, contrary to Tattersfield's results, they were found to be only slightly toxic.

8. It is considered that in view of the fact that *Tephrosia vogelii* grows both widely spread and vigorously in East Africa it should be able to replace the use of nicotine almost entirely. On a small scale home-made solutions could be easily and cheaply prepared, and the extraction of crude tephrosin on a larger scale is also worth consideration.

9. As a paraffin spray against *Antestia* and *Lygus*, *Tephrosia vogelii* extracts proved useless compared with pyrethrum, but as a domestic spray against flies, mosquitoes, etc., paraffin extracts were found to be as effective as pyrethrum and should be able to replace it, at least for local use.

10. The finely powdered pod of *T. vogelii* has some value as an insect powder, due probably to the tephrosal in it, but cannot compare with pyrethrum powders.

11. The seeds contain 12 per cent. of fixed oil from which a soft soap

can be made, suitable, according to preliminary tests, for a spreader but not for domestic purposes. The oil is a non-drying one.

12. Further work on many of these points is continuing.

In conclusion the author wishes to thank Mr T. W. Kirkpatrick for entomological advice and information, and for testing the paraffin sprays against *Antestia* and *Lygus* in the field: and Mr P. J. Greenway for botanical information and for bringing to his notice many of the plants tested during this work.

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THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). I.

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(With Plate XXXIII.)

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INTRODUCTION.

The increase in the demand for insecticidal pyrethrum flowers has led to attempts to establish new centres of production. At present, most of the world supply of the flowers comes from Japan and Dalmatia, but the plant is cultivated on a smaller scale in France, Italy, North Africa and in Kenya Colony. Experimental cultivation of the crop is also in hand in many other countries and it has been shown that the plant will thrive in the temperate climate of England, and will produce good yields of flowers with a high poison content. Little is known, however, of the effect of soil or other environmental conditions upon the growth of the plant and the production of flowers, or of the factors which influence the poison content of the flowers. The possibilities of increasing the yield and toxic value of the flowers, and of prolonging the life of the plant by the application of manures are of economic importance.

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[1] Being part of a thesis approved by the University of London for the Degree of Doctor of Philosophy.

The plant is a perennial and has been successfully cultivated on poor land. In Japan it is the custom to apply manure to the beds before the seeds are sown, and again at later stages in the life of the seedling, but the excessive use of a nitrogenous fertiliser is said to result in increased vegetative growth, at the expense of flower production. The plant there is grown on the hill slopes, and withstands severe winter conditions. Gnadinger, Evans and Corl (3) have shown that in Colorado little loss results from subjecting the plant to winter temperatures as low as −39° C. It is known that pyrethrum will thrive in a warm dry climate, and it is said to prefer a light calcareous soil, while wet, acid or heavy soil conditions have been regarded as unfavourable.

The elucidation of the chemical nature of the active principles of the flowers by Staudinger and Ruzicka (6), followed by the elaboration of chemical methods of assessing the toxicity of the flowers by Gnadinger and Corl (1) and Tattersfield and his co-workers (8), has made possible the investigation of the effect of soil and other conditions upon the insecticidal value of the flowers. The present work has been carried out with the object of defining more accurately the soil conditions under which the plant will flourish for a number of years and produce good yields of flowers of high quality.

Tattersfield (*loc. cit.*) has stated that there is a fairly close correlation between the pyrethrin I content and the toxicity of pyrethrum, and this has been confirmed by other investigators (5, 9) and substantiated by us in recent work. The relative activities of the flowers from plants grown in heavy soil under different manurial treatments have, therefore, been assessed by the determination of pyrethrin I. A more extended experiment using the field plot technique on light sandy soil is in progress, and will be described later.

THE EFFECT OF SOIL FERTILITY.

Fertiliser experiment, carried out in large pots.

This was commenced in 1929, using heavy soil from Broadbalk Field, Rothamsted, which had not been manured for many years. The soil used, which was neutral in reaction, gave the following figures on analysis, expressed on dry matter:

Carbon	1·85 per cent.
Nitrogen	0·17 ,,
Potash as K_2O	0·37 ,,
Phosphate as P_2O_5	0·14 ,,
Potash soluble in citric acid ...	0·012 ,,
Phosphate soluble in citric acid	0·012 ,,

The soil was mixed with 10 per cent. of sand, and weighed amounts of the fertilisers incorporated. The manurial treatments used were:

No manure.
Farmyard manure.
Complete dressing of artificial fertilisers.
Complete artificials, excluding potash.
,, ,, nitrogen.
,, ,, phosphate.
,, ,, chalk.

Eighteen replicates were taken for the unmanured series, twelve replicates each for the farmyard manure and the complete artificial fertiliser treatments, and six replicates each for the nitrogen, potash, phosphate and chalk deficiency treatments.

The complete dressing of artificial fertilisers used per pot of 44 lb. of soil consisted of:

Potassium sulphate	5 gm.
Sodium nitrate	10 ,,
Superphosphate	10 ,,
Calcium carbonate	30 ,,

The farmyard manure was applied at the rate of 400 gm. of fresh manure per pot, equivalent to 20 tons per acre, while the sodium nitrate was added as fractional dressings in aqueous solution. A small amount of manganese (0·5 gm. per pot) was added (as sulphate) in most cases, but the soil contained sufficient of this element to preclude the possibility of deficiency.

The plots were plunged in a raised bed at the Plant Pathological Laboratory of the Ministry of Agriculture, and the whole series was planted early in June 1929 with rooted shoots from a single large plant of vigorous growth, thus avoiding the variable factors that would have been introduced if seedlings had been used. The pots were placed 18 in. apart, the positions in the bed being randomised. The plants were provided and the cultural work undertaken by the staff of the Ministry's Laboratory. A first harvest of flowers was obtained in 1930.

1930 *harvest.* The flowers from each plant were taken on one day, the majority being in the fully open condition. The stalks were completely removed, the heads dried in the shade in a warm glasshouse, and the yield from each plant obtained. The pyrethrin I percentage of the flowers from each plant was then determined.

1931 *harvest.* A separate investigation had shown that the maximum pyrethrin content occurs when the flowers are in the fully open condition. In harvesting the 1931 crop, the flowers were therefore taken from each plant as they came to maturity. A flower showing at least the first peripheral circle of disc florets completely open was regarded as a fully open flower. No differences, however, were seen in the time or rate of maturation of the flowers from the plants subjected to the various manurial treatments. The pyrethrin I content of the flowers from each plant was determined as before.

1932 *harvest.* The flowers from each plant were harvested when fully open, dried and weighed, and a representative sample taken for each manurial treatment for the determination of pyrethrin I.

From the weights of flowers yielded by the individual plants, the mean value, with the standard deviation of the mean, was calculated for each treatment used. These figures, together with the mean values for pyrethrin I, with their calculated standard errors where possible, are given in Table I.

Table I.

Results of fertiliser experiment commenced in 1929.

Treatment	Weight of flowers per plant gm.	Average weight of flowers gm.	Pyrethrin I % of flowers
	1930 harvest.		
No manure	18·18 ±2·32	0·128	0·96 ±0·022
Farmyard manure	24·53 ±2·56	0·135	1·01 ±0·023
Complete fertilisers	24·30 ±3·10	0·129	0·94 ±0·032
No potash	28·18 ±3·90	0·134	1·00 ±0·010
No nitrogen	21·81 ±4·04	0·134	1·01 ±0·021
No phosphate	19·84 ±5·32	0·127	0·96 ±0·017
No chalk	18·05 ±3·00	0·140	1·03 ±0·028
	1931 harvest.		
No manure	34·96 ±2·41	0·118	0·96 ±0·031
Farmyard manure	35·31 ±4·31	0·121	0·96 ±0·019
Complete fertilisers	35·87 ±3·40	0·118	0·99 ±0·014
No potash	45·02 ±5·82	0·112	1·02 ±0·017
No nitrogen	27·98 ±5·73	0·114	0·97 ±0·009
No phosphate	41·41 ±5·69	0·115	1·01 ±0·013
No chalk	37·81 ±3·82	0·120	0·98 ±0·011
	1932 harvest.		
No manure	17·23 ±1·75	0·134	0·88
Farmyard manure	15·94 ±2·34	0·132	0·87
Complete fertilisers	19·49 ±2·50	0·132	0·87
No potash	13·44 ±2·76	0·115	0·80
No nitrogen	19·29 ±1·09	0·131	0·84
No phosphate	20·65 ±2·69	0·127	0·86
No chalk	19·51 ±2·07	0·124	0·80

There were large differences in the weights of flowers yielded by individual plants, and these differences are reflected in the standard errors recorded. For the first year of the experiment a statistically significant increase in the yield of flowers over the yield from the unmanured series was obtained from the treatment where nitrogen and phosphate were supplied with omission of potash. This increase in yield. however, was not maintained in the second and third years of the experiment, when the plants grown in the unmanured soil gave a mean yield of flowers not significantly different from the yields obtained from the plants grown with the application of fertilisers.

For the 1930 and 1931 harvests, the pyrethrin I content of the flowers from the majority of the plants remained remarkably constant and approximated to 1 per cent. of the flowers. There was no significant difference in the mean values obtained for the treatments used. The average figures for pyrethrin I in 1932 were slightly lower than those of the previous years, but were still of a high order.

From the beginning of the experiment seven plants out of the sixty-six appeared to be exceptional. These in 1930 and again in 1931 gave values for pyrethrin I of 0·5–0·6 per cent., the content given by the remainder of the plants being of the order of 1·0 per cent. of the flowers. The exceptional plants did not show the normal vigorous growth exhibited by the majority of the experimental plants, and died before the harvest of 1932. The relatively low pyrethrin I content of the flowers would seem to be associated with the lack of vitality of the plant. The plants giving low values for pyrethrin I were omitted in compiling the final tables of average figures for the fertiliser treatments. The experiment was not continued after 1932, since at this stage some of the plants showed signs of failure.

A second fertiliser experiment was commenced in 1930, using again the heavy Rothamsted soil, and precisely similar manurial treatments, manganese, however, being omitted. Ten replicates were taken for the unmanured series, and eight replicates each for the remaining treatments.

In this case, however, the parent plant selected gave rooted shoots yielding flowers of pyrethrin I content approximating to 0·5 per cent. Pyrethrin I determinations were made on the flowers from each plant in 1931, and a representative sample of flowers from each treatment was analysed in 1932. The mean values, together with the standard errors, calculated for the fertiliser treatments for the first two harvests are given in Table II.

As in the previous experiment, an initial increase in the yield of flowers that was just statistically significant was recorded where nitrogen and phosphate were applied alone, but again this result was not shown in the second year of the experiment.

Table II.

Results of fertiliser experiment commenced in 1930.

Treatment	Weight of flowers per plant gm.	Average weight of flowers gm.	Pyrethrin I % of flowers
	1931 harvest.		
No manure	27·59 ± 2·59	0·170	0·39 ± 0·015
Farmyard manure	29·18 ± 3·44	0·167	0·40 ± 0·013
Complete fertilisers	28·96 ± 1·57	0·159	0·38 ± 0·008
No potash	35·33 ± 1·76	0·161	0·37 ± 0·011
No nitrogen	27·19 ± 1·82	0·167	0·39 ± 0·011
No phosphate	31·75 ± 2·42	0·162	0·40 ± 0·008
No chalk	34·84 ± 2·50	0·166	0·40 ± 0·011
	1932 harvest.		
No manure	17·03 ± 2·19	0·183	0·58
Farmyard manure	16·35 ± 1·68	0·170	0·50
Complete fertilisers	13·71 ± 1·45	0·152	0·54
No potash	16·74 ± 1·83	0·160	0·57
No nitrogen	15·43 ± 3·92	0·163	0·58
No phosphate	16·84 ± 2·20	0·160	0·58
No chalk	18·90 ± 3·31	0·164	0·55

The mean figures for the pyrethrin I content of the flowers were slightly higher in 1932 than those obtained for the 1931 crop. In each year, however, the flowers from the plants grown in the unmanured soil were significantly as rich in pyrethrin I as those from the plants in the manured series. The results tend to show, as before, that the application of manure has but little effect upon the pyrethrin I content of the flowers. The experimental bed of plants as they appeared just before the 1931 harvest is shown in Plate XXXIII.

The climatic conditions during the experimental period may be briefly summarised. A warm dry period which prevailed from the time of planting until September 1929 was followed by a mild period in which the rainfall and sunshine totals were above the average figures. The year 1930 was one of normal weather conditions, with normal sunshine, and with the rainfall evenly distributed throughout the year. The feature of 1931 was the deficiency in sunshine hours, while the temperature was often below normal. The summer months were cold and wet. The early months of 1932 were cold and dry, followed by phenomenal rainfall in May, and a drought in June, accompanied by subnormal sunshine.

Sand-culture experiment.

The yields and pyrethrin I contents of the flowers from the plants grown in the unmanured series of the experiments just described were high, and suggest that the poor soil used was rich enough to meet the manurial requirements of the plant. An experiment to test definite deficiency treatments was therefore carried out, rooted shoots from one parent plant being grown in silver sand in glazed pots to which known quantities of fertilisers were added in solution in water.

The amounts of nutrients supplied to the fully manured plants were as follows:

Sodium nitrate	2·0 gm. per pot
Disodium phosphate	0·56 ,,
Potassium sulphate	0·41 ,,
Calcium chloride	0·10 ,,
Magnesium sulphate	0·28 ,,
Trace of iron and manganese	

Other pyrethrum plants were grown under conditions of nitrogen, phosphate and potash deficiency by the addition, in turn, of one-fifth of the amounts of sodium nitrate, disodium phosphate, and potassium sulphate supplied to the fully manured plants. Four replicates were used in each treatment. The majority of the plants made good growth, and gave flowers of high average weight (0·09–0·19 gm.). The pyrethrin I contents of the flowers from each treatment were determined and are given in Table III.

Table III.

Plants grown in sand with deficiencies of nitrogen, phosphate and potash.

Treatment	Pyrethrin I % of flowers
Fully manured	0·35
Nitrogen deficiency	0·35
Phosphate deficiency	0·33
Potash deficiency	0·34

It is evident that, as far as this experiment indicates, there is no effect of the fertiliser constituents upon the pyrethrin I content of the flowers.

THE PYRETHRIN CONTENT OF THE FLOWERS FROM YEAR TO YEAR.

Differences may occur in the type of flowers produced by plants of *Chrysanthemum cinerariaefolium.* The flowers of some plants bear short ray florets, and these also appear in the flowers resulting from rooted shoots from these plants. Certain plants have been observed to give flowers maturing at an earlier or later date than the majority of the experimental plants, and this relative earliness or lateness of flowering is again exhibited in plants derived from these individuals by vegetative propagation.

Large variations were noticed in the percentage of pyrethrin I and of the total pyrethrins in the flowers from different plants. We have found, however, that a given plant has yielded flowers containing approximately the same level of percentage of pyrethrin I from year to year when observations were made for at least three successive years (see Table IV). This approximately constant degree of richness in pyrethrin I of the flowers of a given plant appeared to be independent of the number of flowers produced each year. The total pyrethrin contents of the fully open flowers from eight individual plants were determined for two successive harvests, and approximately constant values were obtained for each plant (see Table V). The determination of the total pyrethrins in the flowers from these plants was not carried out after the second year. We are therefore unable to say whether the pyrethrin II found in the flowers of a given plant remains of the same order from year to year, as is the case with the pyrethrin I.

Table IV.

Fluctuations in pyrethrin I percentage of flowers from year to year.

	Pyrethrin I % of flowers			
Plant	1929	1930	1931	1932
EM 1	0·32	0·30	0·28	—
2	0·45	0·52	0·55	—
3	0·51	0·60	0·55	—
4	0·29	0·27	0·16*	—
5	0·30	0·28	0·22*	—
6	0·40	0·42	0·55	—
G 4	1·00	1·06	0·80†	0·91†
G 9	0·64	0·67	0·56†	0·59†
F 11	1·16	1·10	0·92†	0·88†

* Flowers were overblown.

† Rooted shoots from parent plants. The flowers from rooted shoots from G 4 and F 11 in 1933 gave 0·86 and 1·00 per cent. of pyrethrin I respectively.

Table V.

Total pyrethrins percentage of the flowers for two successive years.

	1929		1930	
Plant	Pyrethrin I	Total pyrethrins	Pyrethrin I	Total pyrethrins
G 4	1·00	1·98	1·06	2·10
G 5	0·74	1·47	0·63	1·41
G 7	0·53	1·27	0·53	1·42
G 8	0·95	1·80	0·97	1·84
G 9	0·64	1·78	0·67	1·81
G 10	0·30	0·96	0·30	0·79
G 12	0·67	1·47	0·66	1·58
F 11	1·16	2·02	1·10	2·08

DISTRIBUTION OF THE PYRETHRINS IN THE DIFFERENT PARTS OF THE FLOWERS.

Fully open flowers, grown in England from Japanese seed, and having at least one-quarter of the disc florets open and no parts missing, were dissected into the constituent fractions, petals, receptacles and involucral scales, ovaries and the remainder of the disc florets. The percentage weight of each fraction in the flowers was ascertained, and the total pyrethrin contents of the fractions were determined by the micro-reduction method (4). The percentage distribution of the pyrethrins in the different parts of the flowers was then calculated, and is shown in Table VI.

Table VI.

Distribution of the pyrethrins in fully open flowers.

	Composition of flowers	Total pyrethrins
	%	%
Petals	25·2	0·18
Receptacles and involucral scales	20·4	0·27
Disc florets excluding ovaries	31·4	0·48
Ovaries	23·0	4·54

The sample from which the flowers were taken gave on analysis a total pyrethrin content of 1·37 per cent. The total pyrethrin content calculated from the figures obtained by the analysis of the constituent fractions was 1·30 per cent.

The ovaries were found to be much richer in pyrethrins than the remaining parts of the flowers. Of the total pyrethrins found in the fully open flowers examined, 80 per cent. was located in the ovaries, and about 10 per cent. in the remainder of the disc florets. Thus the disc florets of the flowers, made up of the corolla and stamens with the attached inferior ovaries, contained 90 per cent. of the pyrethrins found in the flowers.

The percentage of pyrethrin I in the ovaries taken from flowers that had just reached the fully open condition was determined. A value of 1·69 per cent. was obtained, while the average weight of the ovaries was 0·21 mg. The ripe fruits from the overblown heads, however, gave a value for pyrethrin I of 0·38 per cent., while the average weight of the seeds had increased to 0·80 mg. During the ripening of the ovaries there is, therefore, a fourfold increase in weight, while the poison content falls proportionately. This result confirms earlier work (7), when it was shown that the *percentage* pyrethrin content of the flowers falls after fertilisation due to increase in the weight of the heads without a significant change in the absolute amounts of the pyrethrins present in the flowers.

DISCUSSION OF RESULTS.

An increase in the weight of the heads that was just significant was obtained in the first year of each manurial experiment from the treatment where nitrogen and phosphate were applied alone, but this result is not of practical importance. After the first year of the experiments, there were no significant differences in the yields of flowers from the plants grown in the unmanured and enriched soils. No differences due to the manurial treatments used were observed in the time or rate of maturation of the flowers.

At no stage in the experiments was a significant difference found in the pyrethrin I content of the flowers from plants in the unmanured series and in the fertilised series. In the sand-culture experiment, the plants grown under conditions of nitrogen, phosphate and potash deficiency gave flowers of pyrethrin I content equal to that of the flowers from the fully manured plants.

The data obtained show that when pyrethrum is grown on heavy soil under pot-culture conditions, the pyrethrin I content of the flowers is not influenced to any great extent by the application of manures. It appears that the plant will grow quite well on poor soil, and will produce flowers having a high poison content. In this connection, it is of interest that an experimental plot of pyrethrum at Harpenden, laid down in 1926 upon heavy soil to which no manure had been applied, has yielded rich flowers each year since 1927. A good crop was obtained in 1933, when the soil must have been in a low state of fertility.

It was hoped, as a result of the experiments carried out on the growth of pyrethrum in poor soil to which known amounts of fertilisers were added, to obtain information on the effect of soil fertility conditions upon the length of the life of the plant. The pot technique employed, however, proved unsatisfactory for this purpose, as, although the pots used were large, the root development of the plants was so considerable that the experiment could not be continued after the third year.

In order to determine the full effect of fertilisers upon the plant, particularly with regard to the number of years the plant will continue to give flowers, the yield and pyrethrin content of the flowers, an experiment is in progress on a poor sandy soil using the small plot technique, and will be reported upon later.

The data presented indicate that the extent to which the pyrethrins are produced in the flowers is characteristic of a particular plant, and that it is not influenced to any extent by the enrichment of the soil.

Earlier work by Gnadinger and Corl (2) and Tattersfield (7) has shown that the pyrethrin content is at a maximum when the flowers are fully open. It is possible, however, that the flowers from one set of plants, at an early stage in their development, might prove richer than fully open flowers from another set of plants.

Although the genetics of pyrethrum are too complex to make any definite statement possible, the extent to which the pyrethrins are produced in the flowers appears to depend upon an inherent property of the plant in question. A plant initially producing flowers rich in pyrethrins has, with us, continued to produce flowers of a similar degree of richness for a number of years, and rooted shoots from the parent plant have again yielded rich flowers (see Tables IV and V, p. 677).

The average pyrethrin content of the flowers from a given bed of plants will depend upon the relative numbers in the bed of plants producing rich and poor flowers. A crop of flowers rich in pyrethrins might be secured by a careful selection of plants in the early stages of cultivation according to their virility and pyrethrin-producing power, or by the employment of cuttings from the roots of rich plants. We have not, however, up to the present time, been able to correlate the pyrethrin content of the flowers with any particular morphological characteristic of the plant, so that the process of plant selection, based upon chemical evaluation of the flowers of individual plants, is likely to be laborious. The number and size of flowers borne by a plant have an economic importance almost as great as the content of active principles, but whether there is a strict correlation between these factors is unknown.

The greater part of the pyrethrins of the fully open flowers are located in the ovaries. This confirms the result of Gnadinger and Corl (2). Nothing is known of the physiological rôle of the pyrethrins in the plant. The fact that there is no significant loss in their absolute amount after fertilisation suggests that they are not further metabolised in the flowers. We have no conclusive evidence to show that the pyrethrins are formed as waste products in the anabolic processes leading to the final maturation of the flowers, or that they play an important part in the subsequent metabolism of the seed.

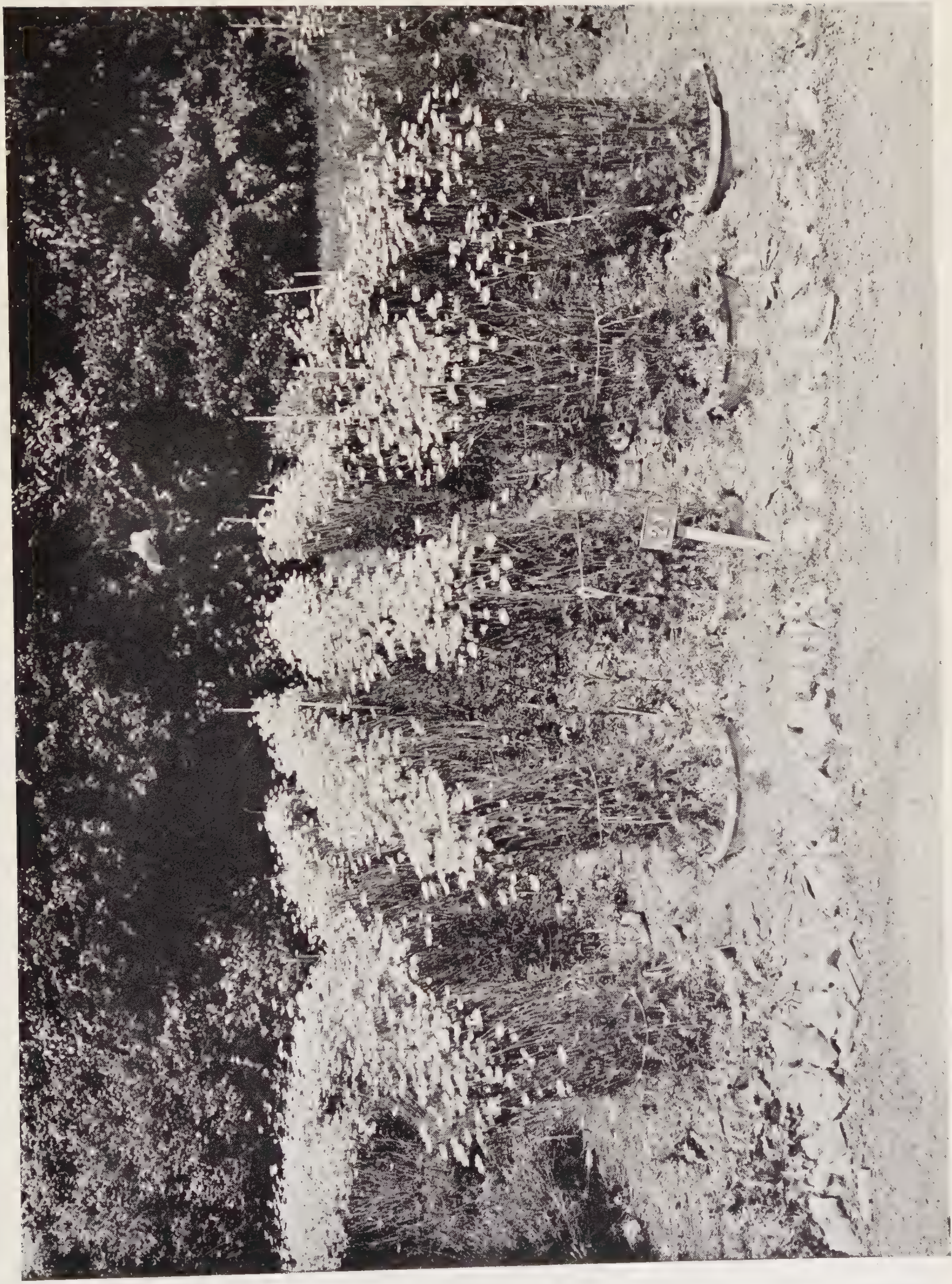

Fig. 1

MARTIN AND TATTERSFIELD.—THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). I (pp. 670–681).

Summary.

1. The effect of soil fertility upon the insecticidal value of the flowers has been studied in a series of pot experiments. On heavy soil the pyrethrin I content of the flowers was not increased by the application of fertilisers. The plants produced good yields of flowers, rich in pyrethrins, when grown in soil of low fertility.

2. Under conditions of normal growth and vitality, the extent of production of the pyrethrins in the flowers was characteristic of the individual plant and was dependent upon some factor which appeared to be genetical in character. A plant initially producing flowers of high or low percentage poison content continued to give flowers of the corresponding degree of richness in succeeding years, independently of the application of manures, or apparently of the number of heads produced. Plants derived from rooted shoots produced flowers corresponding in quality with those initially yielded by the parent plant. The insecticidal value of pyrethrum flowers may be improved by plant selection, followed by vegetative propagation.

3. In the fully open flowers, the complete disc florets contained 90 per cent. of the total pyrethrins present in the flowers, and of this the greater part was found to be located in the ovaries.

We desire to express our most sincere thanks to Mr J. C. F. Fryer and Mr C. T. Gimingham of the Plant Pathology Department of the Ministry of Agriculture for the facilities placed at our disposal for carrying out these experiments, for the raising of the plant material used and for the care of the plants during the experiments.

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EXPLANATION OF PLATE XXXIII.

Fig. 1. Experimental bed of pyrethrum plants as they appeared just before the 1931 harvest.

(*Received March* 1*st*, 1934.)

THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). II.

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(With Plates XXXIV and XXXV.)

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Introduction.

In the preceding communication, the effect of soil fertility conditions upon the growth of pyrethrum (*Chrysanthemum cinerariaefolium*), and the pyrethrin content of the flowers was described. It was found that the plant will thrive on heavy neutral soil of low fertility, and that under the conditions of the experiment the pyrethrin I content of the flowers was not influenced by the application of manures. The factor governing the poison content of the flowers appeared to be genetical in character.

Other environmental conditions, however, are likely to affect the extent of production and the insecticidal value of the flowers. Of these, temperature, rainfall, the amount of sunlight, and the length of dormant period during the winter months appear to be the most important.

Pyrethrum will grow well and produce flowers of high insecticidal activity in regions of high altitude in Kenya Colony, although the flowering period is much extended, while in the lower regions, where a higher temperature prevails, the morphological characters are changed and smaller yields of flowers are obtained. Moreover, in a strictly tropical belt below a certain altitude, where the plant continues to grow throughout the year, no flowers are produced. Gnadinger (1) reports that the experimental planting of pyrethrum in Kentucky was unsuccessful owing to the mild winter failing to induce a dormant condition, with subsequent injury due to sudden cold periods. The preclusion of sunlight during growth was shown by Gnadinger, Evans and Corl (2) to result in a reduced pyrethrin content of the flowers, but the flowers in this case were abnormal and small.

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[1] Being part of a thesis approved by the University of London for the Degree of Doctor of Philosophy.

The present investigation had as its object the study of the effects of relatively high temperatures, partial shading, and of dormancy during the winter months upon the growth of pyrethrum and the production of flowers. Pyrethrin determinations have been made on the flowers from the experimental plants by the method of Tattersfield, Hobson and Gimingham (3).

EXPERIMENTAL.

In a preliminary experiment, fourteen rooted shoots of equal size and root development, from one parent plant, were grown in large pots in poor heavy soil, to which 10 per cent. of sand and a basal dressing of fertilisers were added. After 12 months' growth, the fourteen plants were randomised, and subjected to the following treatments:

Control plants. These were kept in the open under the normal climatic conditions prevailing over the experimental period.

Plants grown at a higher temperature. These were kept in a glasshouse heated during the winter months, and maintained at 15–25° C.

Partially shaded plants grown at the higher temperature. These were kept in the glasshouse as before, but were allowed half the normal amount of sunlight.

The shading of the plants was effected by means of beaver-board boxes darkened on the inside. The plants were covered and exposed alternately each day at noon. In this way, differences in the value of morning and afternoon sunlight were eliminated. The amounts of rain falling upon the control plants were recorded, and were taken into account in supplying water to the glasshouse plants.

The experimental treatments were commenced in February 1931. When near the time of flowering, some of the glasshouse plants were attacked by mites, but were successfully treated with colloidal sulphur. The flowers were taken as they reached the fully open condition.

The plants grown at 15–25° C. in full sunlight were the first to flower. The flowering period extended from the beginning of April until the beginning of June 1931, and showed a peak at the end of April. The shaded plants at 15–25° C. commenced to flower later, and flowered over a shorter period. The flowering of the control plants commenced at an even later date, the flowering period was short, and the yield of heads was greatly in excess of the yields from the glasshouse plants. There was a pronounced falling off in the average weight of the heads as flowering proceeded. The flowers were analysed for pyrethrin content by the acid method, or, where the weight of the flowers obtained was insufficient, by the micro-reduction method. The average figures for each treatment are given in Table I, and it will be seen that the differences between the treatments are sharply defined.

Table I.

The effect of summer temperature and shading upon pyrethrum.

	Control plants at 2–15° C.	Plants at 15–25° C.	Shaded plants at 15–25° C.
Flowering period in days	22	56	30
No. of flowers per plant	106	82	33
Weight of flowers per plant, gm.	14·37	5·91	1·29
Average weight of flowers, gm.	0·136	0·072	0·039
Pyrethrin I per cent. of flowers	0·42	0·36	—
Total pyrethrins per cent. of flowers	1·65	1·25	0·94*
Pyrethrins formed per plant, gm.	0·237	0·074	0·012

* By micro-reduction method.

The growth of the plants at a higher summer temperature than that to which the control plants were subjected is seen to result in a longer flowering period, and reduction in the number, average weight and pyrethrin content of the flowers formed. The effect of shading at the higher temperature is further to reduce the number of heads given, their average size and pyrethrin content. The differences in pyrethrin content of the flowers are clearly seen when the amounts of pyrethrins, in grams, formed per plant and located in the flowers are compared.

The glasshouse plants, from which the flower stalks and mature foliage were removed after the last of the experimental plants had flowered, were kept unshaded in the glasshouse at 15–25° C. through the winter of 1931–2. The plants continued to grow well, but no flowers were produced in the summer of 1932. Morphological changes were noticed: the leaves were originally small and deeply pinnatifid, but new leaves produced during growth at the higher temperature were much larger and less deeply pinnatifid. Three of the glasshouse plants, taken at the normal time of flowering in June 1932 after continuous growth for twelve months, are seen in Plate XXXIV, fig. 1. The foliage was taken in July 1932, air-dried, and analysed for pyrethrin I. A value of 0·04 per cent. was obtained. Thus the foliage of the plants which produced no flowers had no insecticidal value.

A second experiment was commenced in 1931 in which the effect of shading the plant at normal temperatures and the effect of dormancy were studied. Twelve rooted shoots from each of two parent plants (A and B) were grown as before in large pots in heavy soil. Each set of twelve plants was divided at random into four groups of three plants each. Of these, two groups spent the winter of 1931–2 exposed to the normal climatic conditions, while the remainder were kept in the glasshouse at 15–25° C. Partial shading, carried out as in the previous experiment, of half the plants in the open and half the glasshouse plants was commenced in the spring of 1932. The addition of water to the glasshouse plants and the outside shaded plants was controlled in accordance with the amount of rain falling upon the unshaded plants.

The plants grown under the normal climatic conditions in both the unshaded and shaded series gave flowers, but as the plants were young the numbers of heads were small. The flowers from each plant were taken as they reached maturity, air-dried and weighed, and pyrethrin I determinations made. The plants in the glasshouse showed vigorous growth with the formation of large leaves, but with the exception of two plants, which formed a single small head each, no flowers were produced.

The average figures obtained for the plants from A and B grown under normal climatic conditions with and without partial shading are given in Table II. The chief effect of shading is seen to be the reduction in the average weight of the flowers, while the pyrethrin I content is also lowered.

Table II.

The effect of shading at normal summer temperature.

	Unshaded plants		Shaded plants	
	A	B	A	B
No. of flowers per plant	23	20	30	10
Weight of flowers per plant, gm.	4·92	4·66	3·29	1·64
Average weight of flowers, gm.	0·214	0·233	0·110	0·164
Pyrethrin I per cent. of flowers	0·48	—	0·43	—

The plants produced flowers to the normal extent only when they had been allowed a dormant period during the preceding winter. It was therefore decided to bring some of the outside plants into the glasshouse before the winter of 1932–3 and again in the spring of 1933, and also to subject some of the glasshouse plants to the outside conditions, with the object of observing the effects of dormant and non-dormant conditions upon subsequent flower production. The foliage formed during the previous year was removed from all the plants and only the young foliage retained. The plants in the glasshouse and outside were divided at random into three groups of four, two plants in each group being derived from the parent plant A, and two from B. The outside plants were treated as follows: one set of four was taken into the glasshouse at the end of July 1932, a second set was removed inside during March 1933, and the remaining four plants were allowed to remain outside under the normal climatic conditions. Similarly, one set of four glasshouse plants was taken outside in July 1932, one set during March 1933, and the third set was retained in the glasshouse. The plants were allowed gradually to acclimatise themselves to their new conditions.

The plants which had spent 1931–2 under normal conditions and which had flowered in 1932 continued to grow on removal to the higher temperature conditions, and did not experience a dormant state during the winter of 1932–3. A few flowers were produced by each plant in June 1933. The effect of the higher temperature immediately before flowering is seen in the second set of outside plants which were moved into the glasshouse in the spring of 1933, after a winter dormant period. Here large numbers of flowers were produced, but the flowering was hastened, all the flowers being in the fully open condition early in May 1933. The control plants, *i.e.* those which had a dormant period in 1931–2, and again in 1932–3, and which were allowed to flower in 1933 under normal climatic conditions, gave in June rather fewer flowers than the preceding series, but the heads were of a higher average weight.

The four plants which had not experienced a dormant period during the winter of 1931–2 resulting in no flower production in 1932, and which were returned to the normal climatic conditions in July 1932, gave large numbers of flowers in 1933, apparently the effect of a dormant period during the preceding winter. On the other hand, the plants which had no dormant period either during 1931–2 or 1932–3, but which were allowed the normal summer conditions in 1933, gave no fully open flowers, but merely a few buttons, none of which reached maturity. The plants which had spent the whole of the experimental period at 15–25° C. gave a few fully open flowers in 1933, but this may have been due to the fact that they were situated in the glasshouse near a ventilator. The average numbers of fully open flowers that were obtained under these various conditions are given in Table III. The numbers of fully open flowers given do not represent the full extent of the flower production, since flower-heads in the button to half-open condition were present. The average number of these left on each plant after the fully open flowers were taken is given in brackets.

Table III.

The effect of winter dormancy and different summer temperatures upon the flowering of pyrethrum.

Treatment	Series	Flowers per plant	Weight of flowers per plant gm.	Average weight of flowers gm.
Dormant 1931–2, non-dormant 1932–3, high temperature 1933	A	14	1·80	0·129
	B	19	2·13	0·112
Dormant 1931–2, dormant 1932–3, high temperature 1933	A	80	6·37	0·080
	B	105	9·26	0·088
Dormant 1931–2, dormant 1932–3, normal temperature 1933	A	67 (54)	7·12	0·106
	B	49 (97)	6·79	0·138
Non-dormant 1931–2, dormant 1932–3, normal temperature 1933	A	53 (75)	5·45	0·103
	B	52 (112)	6·36	0·122
Non-dormant 1931–2, non-dormant 1932–3, normal temperature 1933	A	(3)	Gave no fully open flowers	
	B	(45)		
Non-dormant 1931–2, non-dormant 1932–3, high temperature 1933	A	5	0·46	0·092
	B	11 (3)	1·04	0·094

Photographs were taken in May 1933, showing the early flowering of the plants which were subjected to the higher temperature after two dormant periods, and again in June 1933, at the normal time for the flowering of pyrethrum in this country (Plate XXXIV, figs. 2, 3 and Plate XXXV, figs. 4–6).

The differences in the appearance of the plants grown without a dormant period and those which, following a dormant period, produced flowers, are clearly seen in Plate XXXIV, fig. 2. Plant No. 2 had no dormant period during the winter of 1932–3, and showed large leaves, while No. 4, kept under the normal climatic conditions throughout the experiment, had small feathery leaves, the greater part of the plant being made up of thick flowering stems. The results indicate that under conditions which induce the continuous growth of the plant throughout the year, growth takes place at the expense of the production of flowers.

During the experimental period, the extremes of the temperatures recorded in the glasshouse were 11 and 30° C. The average monthly temperature in the glasshouse during the winter months fluctuated between 16 and 20° C., while during the summer average monthly temperatures of 20–24° C. were recorded. The lowest temperature that the plants under normal conditions experienced was −10° C. on one day each in March 1932 and January 1933. The lowest average monthly temperature recorded (outside screen temperature) was 2° C. in February 1932, and the highest 16° C. in July 1932.

Discussion of results.

Gnadinger, Evans and Corl (2) have studied the effect of growing pyrethrum in full sunlight, partial sunlight, and in the absence of sunlight. When grown in partial sunlight, the numbers of flowers were reduced, while the average weight of the flowers was less than that of the flowers from the plants grown in full sunlight. The plants grown in partial sunlight actually gave flowers richer in pyrethrins than the control plants, but it was concluded that this was due to differences in the pyrethrin-producing power of the individuals used. We have endeavoured to eliminate the factor of individuality by using rooted shoots from a single plant. Gnadinger found that the growth of the plant in the absence of sunlight resulted in relatively few small flowers of low pyrethrin content. We have found that when pyrethrum is grown at a summer temperature of either 15 or 25° C. reduction in the amount of light available to the plant results in smaller flowers of poorer quality. The lowering of poison content may possibly be due to the less intense metabolism on reduction of the light, the plant showing under these conditions less vitality and growth. The illumination reaching the plants kept in the glasshouse would be deficient in light of short wave-length, as this would partially be eliminated in passage through the glass.

The effects of temperature and dormancy upon the production of flowers by pyrethrum are closely interrelated. The influence of a high temperature apparently depends upon the time at which it is in operation. The plants which, following a winter dormant period, were subjected to a high summer temperature, gave large numbers of flowers, but the flowering took place at an early date, while in no case did those which had been subjected to the higher temperature during the winter months produce more than a few flowers in the following summer. As far as our experiments show, the production of flowers in the summer is dependent upon dormancy in the preceding winter only, since the plants which, after active growth during the winter of 1931–2, were exposed to cold winter conditions in 1932–3, produced flowers in the summer of 1933. The type of foliage formed by the plants grown under the higher temperature conditions was associated with the inability to produce large numbers of flowers. The few flowers formed by these plants were attached to long, straggling and weak flowering stalks. The normal rapid flowering of pyrethrum thus depends upon sufficient differences in the winter and summer temperatures. The winter temperature must be sufficiently low to induce a reasonably extended dormant period.

The results of these experiments have provided an indication that the pyrethrins are not formed photosynthetically in the leaves with subsequent translocation to the flowers. The foliage of the individuals grown at the higher temperature with non-production of flowers was not found to be richer in pyrethrins than that of the plants which flowered normally. It is reasonable to suppose that if the pyrethrins had been formed in the leaves, then in the absence of flowers the foliage would have been richer in pyrethrins than was actually found to be the case.

SUMMARY.

1. The effects of light, temperature and dormancy upon pyrethrum have been studied. The partial shading of the plant during the five months preceding flowering resulted in the production of smaller flowers with a reduced pyrethrin content.

2. The successful flowering of the plant was largely dependent upon the relative temperatures experienced throughout the year. A dormant period, dependent upon sufficiently low winter temperatures, was shown to be necessary for the normal production of large numbers of flowers.

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EXPLANATION OF PLATES XXXIV AND XXXV.

(Normal time of flowering is end of June.)

PLATE XXXIV.

Fig. 1. Taken June 24th, 1932. Plants at normal time of flowering after twelve months' continuous growth. No flowers formed.

Figs. 2, 3. Taken on May 11th, 1933. Plants 2, 14, dormant 1931–2, non-dormant 1932–3, high temperature 1933. Plants 9, 18, dormant 1931–2, dormant 1932–3, high temperature 1933. Plants 4, 24, dormant 1931–2, dormant 1932–3, normal temperature 1933. Flowers on 4 and 24 in button stage.

PLATE XXXV.

Fig. 4. Taken on May 11th, 1933. Plant 1, non-dormant 1931–2, non-dormant 1932–3, normal temperature 1933. Plant 5, non-dormant 1931–2, dormant 1932–3, normal temperature 1933. Plant 11, non-dormant 1931–2, non-dormant 1932–3, high temperature 1933. Flowers forming on 5.

Fig. 5. Taken June 22nd, 1933. Plants as in Fig. 4, plant 5 in flower, and few flowers on 11.

Fig. 6. Taken June 22nd, 1933. Plant 21, treatment given under 5 in Fig. 4. Plant 23, treatment given under 1 in Fig. 4. Plant 13, treatment given under 11 in Fig. 4. Few flowers shown on 23 and 13.

(*Received March* 1*st*, 1934.)

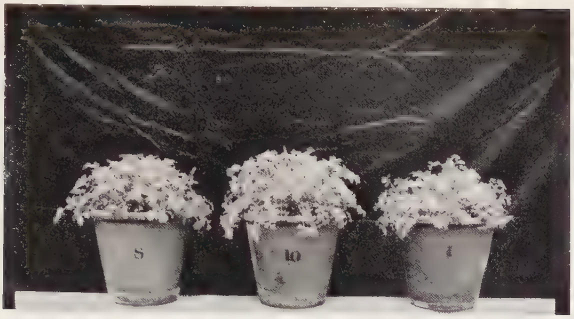

Fig. 1.

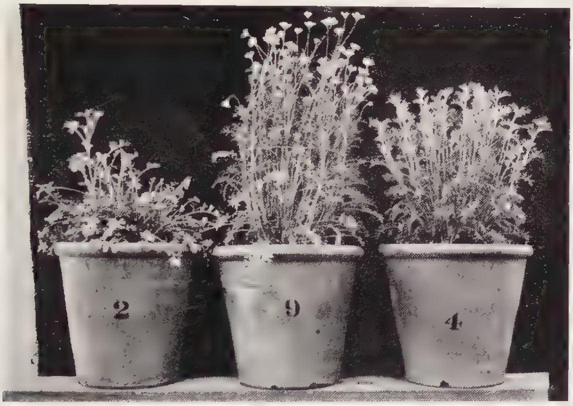

Fig. 2.

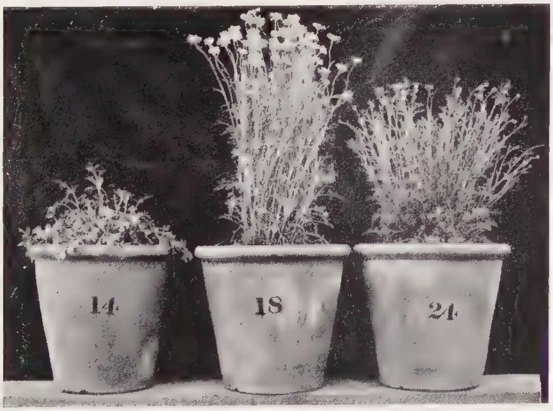

Fig. 3.

MARTIN AND TATTERSFIELD.—THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). II (pp. 682–690).

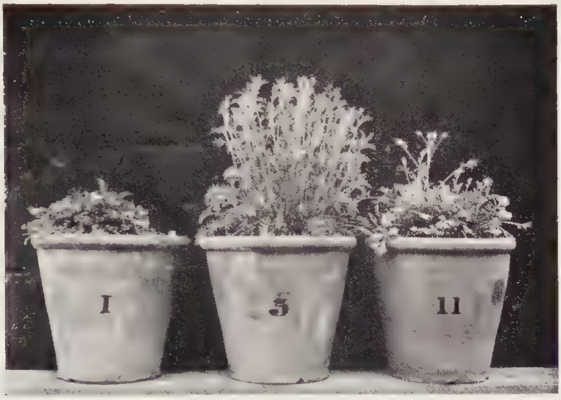

Fig. 4.

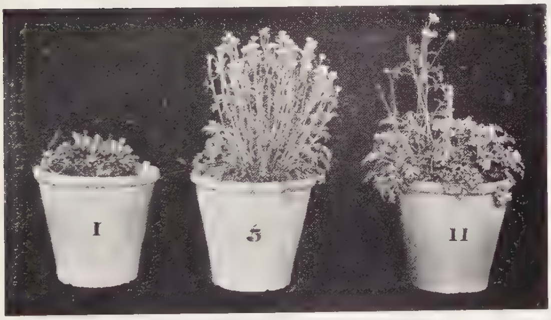

Fig. 5.

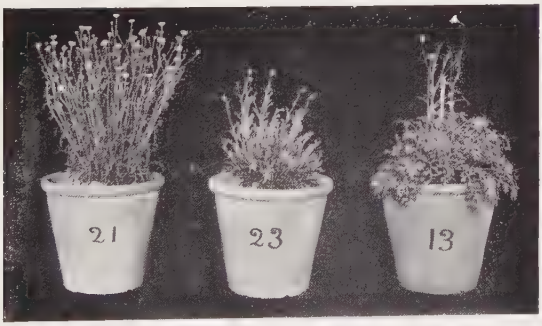

Fig. 6.

MARTIN AND TATTERSFIELD.—THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). II (pp. 682–690).

AN APPARATUS FOR TESTING CONTACT INSECTICIDES.

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(With Plate XXXVI and 5 Text-figures.)

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INTRODUCTION.

In 1924 Tattersfield and Morris (2) published an account of an apparatus suitable for spraying insects with contact insecticides under laboratory conditions. This apparatus has been in use since that date and has proved satisfactory for its purpose. The atomiser used in this model was so constructed that a current of air was blown over the end of a capillary tube dipping into a reservoir containing the solution or suspension of the insecticide to be tested; the fluid was drawn from the reservoir and thrown into a fine spray over the insects. The data given in the previous paper showed that the sprayings could be replicated with considerable exactitude, and insecticide tests carried out gave sigmoid curves, relating concentration of poison to percentage number of insects rendered moribund or dead, now regarded as typical for the relation of dosage and effect. The distribution of the spray over the area upon which the insects were placed was comparatively uniform at the depth of the dish from the nozzle used. When, however, replicates of the atomiser had to be constructed it was found no easy matter to make the necessary adjustments between the orifices through which the air was blown and that of the tube connected with the reservoir. Although this type of atomiser, in which each minute drop, as it emerges, is scattered by an air blast at right angles to the path of the drop, results in considerable evenness in the distribution of the spray, it unfortunately affords no opportunity for regulating the fineness of division of the spray or the time required for delivery. Messrs Pellant and Son of Harpenden have recently designed and made for us two types of atomiser, suitable for operation by compressed air, in which the air current dispersing the drop is in line with the path of the latter, as it emerges from the orifice of the tube connected with the reservoir. By the use of a needle valve, the size of the actual spray droplets and the time taken in spraying any given amount can be regulated. This paper gives an account of these new types of atomiser and the distribution of spray given by them.

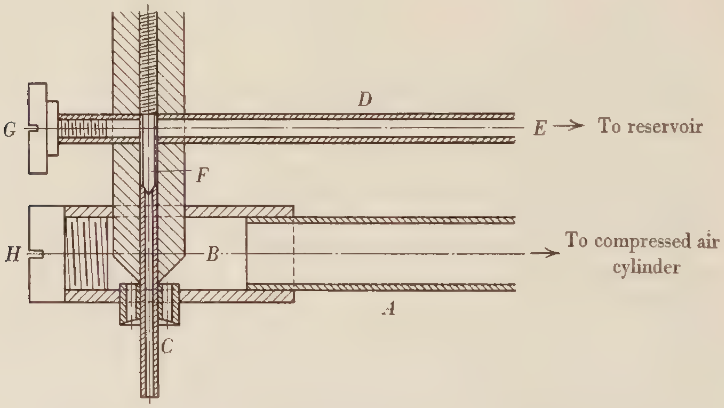

Fig. 1. Vertical section through atomiser No. 1 (twice actual size).

DESCRIPTION OF ATOMISER No. 1.

A plan of atomiser No. 1 is given in vertical section in Text-fig. 1. It consists essentially of a brass tube of narrow bore *D* extended beyond *E* and bent at right angles to dip into a small glass reservoir; at the other end the tube is brazed into a stout brass tube of narrow bore as shown, into the lower end of which a capillary brass tube is brazed; this fine tube can be partially or wholly closed by the needle *F*, worked by a screw with milled head. The extension *G*, fitted with a screw, affords a means of clearing *D* if it becomes clogged, either by blowing air through or by means of a fine wire. The tube *A* which leads from the air cylinder, fits tightly into the chamber *B*, the compressed air passing through three orifices, two of which are shown at *C*. These openings are cut so that the outflow of air rushes as closely as possible along the jet carrying the liquid. The opening closed by a screw at *H* permits of clearing tube *B* by blowing a little air through from the compressed air cylinder.

DELIVERY OF SPRAY FROM TYPE ATOMISER No. 1.

The atomiser is fixed into a clamp, which allows such movements as are requisite for centring the spray. The clamp is attached by pins to the brass plate let into the wooden lid (see Plate XXXVI), which can be made to fit accurately a large glass cylinder 20 cm. in diameter by means of movable blocks. The jar, which is supported on a levelling platform, is fitted inside with a second brass platform with levelling screws and ivory-tipped screws for holding it firmly in a central position. (By means of marks on the jar the platform can always be returned to the same position after removal.) The internal platform can be made to take dishes of different sizes by means of adjustable uprights. The platform and atomiser are so adjusted with respect to each other that the centre of the former falls directly under the tip of the atomiser. A gallows arrangement with adjustable plummet with pointed end is attached to the atomiser; under the pointed end of the plummet is fixed a piece of squared paper, which serves to indicate any movement of the atomiser. The cone of the spray is centred by lowering into the jar a circular glass plate with a central hole through it to facilitate removal; on this glass plate a circular disc, of the size of the dish to be used for spraying, is placed with its centre over the centre of the platform. 1 c.c. of a solution of saponin in water (0·5 or 1·0 per cent.) is pipetted into the reservoir attached to arm *D* of the atomiser. The tube *A* is connected as shown in the plate through a pressure gauge and air filter to a compressed air cylinder, adjusted to deliver air at known pressure. The lid having been placed in the marked position on the jar, the air is turned on and the liquid in the reservoir is thrown in a fine spray over the plate. The operation is repeated with suitable adjustments of the atomiser until the distribution of the spray is symmetrically disposed about the smaller disc. When adjusted, the clamps are screwed up tight, to prevent further movement. As the brass plate contains two pegs fitting accurately through the plate of the clamp bearing the nozzle, the latter can be taken out and replaced if required without greatly modifying the distribution of the spray. This operation, however, should be carried out as seldom as possible and the distribution of the spray re-checked afterwards.

The above procedure enables one to obtain as even a distribution as is usually necessary in spraying technique; for more accurate work, however, the distribution can be gauged by lowering into the jar over the platform a large glass disc, to the lower side of which is attached a sheet of paper ruled in concentric circles. A small dish of known diameter can then be placed in positions at varying distances from the centre and the amount of spray falling upon it determined by weighing.

The data for the weight of spray falling on a dish 19·63 sq. cm. in area when the cone of spray was symmetrically disposed and the needle valve open to different amounts are given in Table I.

Table I.

Amount of spray falling upon dish 19·63 *sq. cm. internal area.* 1 *c.c. water sprayed.*

Distance of jet from dish 31·0 cm. Spray atomiser No. 1.

Valve open	No. of sprayings	Mean weight of spray (gm.)	Standard error
Fully open = 2 mm.	8	0·4445	±0·0006
Half-open = 1·0 mm.	10	0·4461	±0·0012
One-quarter open = 0·5 mm.	5	0·3871	±0·0029

These data show that the cone is comparatively narrow and that little difference is made to the dispersion of the spray by the change of the needle valve from half-open to fully open, although reduction to one-quarter open gives the distribution a slightly wider angle. In actual trial it was found that the amount of liquid delivered is rather too great, and it was evident that means would be needed for scattering the spray more efficiently.

DISTRIBUTION OF SPRAY BY ATOMISER No. 1.

For determining the evenness of distribution of spray the method described was employed. A circular sheet of paper was ruled with concentric circles 2·45 cm. apart, and in addition small circles (the size of the dish to be used for weighing the spray) were described, radiating from the centre at angles of 60°. The paper was attached by suitable adhesive to the underside of the circular glass plate. The spray was centred in the usual way, and the weights of spray falling upon a small glass dish 2·7 cm. external diameter with walls 0·65 mm. deep were determined by weighing. Three solutions were sprayed: (1) distilled water, (2) a half per cent. solution of saponin in water, (3) a half per cent. solution of saponin containing 5 c.c. per 100 c.c. of absolute alcohol (a solution frequently used by us in spraying technique). The needle valve was in the fully open position, that is it was raised 2 mm. The data are given with standard errors in Table II.

Table II.

Weights of spray falling upon a dish 5·725 *cm.*^{2} *in area at a depth of* 31 *cm.*

	Mean weight of spray (gm.) at a distance (cm.) of centre of dish from centre of plate of					
Spray used	0 (centre)	0·67	1·35	2·7	5·4	8·1
Water	0·1293 ±0·0026	—	0·0946 ±0·0037	0·0368 ±0·0022	0·0086 ±0·0005	0·0036 ±0·0006
Saponin solution 0·5 %	0·1242 ±0·0020	0·1159 ±0·0016	0·0968 ±0·0028	0·0533 ±0·0021	0·0097 ±0·0007	—
Saponin solution 0·5 % + 5 % by vol. alcohol	0·1208 ±0·0016	0·1133 ±0·0034	0·0921 ±0·0048	0·0457 ±0·0033	0·0092 ±0·0003	—

Although, as the values for the standard errors show, there was some variation in the weights of spray falling upon the dish in different positions in the same concentric circle, there was no certain reason for considering that this was due to the spray not being symmetrically disposed with respect to the dish, rather than to experimental error. The figures are plotted in Text-fig. 2.

The data in Table II and the curves demonstrate that with this atomiser, although there is only a slight change in the distribution of the spray when saponin solution and saponin solution containing alcohol are used instead of water, there is a tendency to a wider scatter of droplets when the surface tension is reduced. The radius of the dish usually used for spraying purposes is about 2·5 cm. The data and the curves show that this atomiser is *not* eminently suitable for the quantitative spraying of insects scattered at random over an area of about 20 sq. cm., owing to the rapid diminution of the amount of spray deposit with an increase in distance from the centre. The atomiser has, however, been used in certain spray trials and, provided 1 c.c. was sprayed and a large enough number of insects were employed, quantitative comparisons could be obtained. The curves obtained by plotting dosage against kill were *S*-shaped and reduced to a straight line when Bliss's probits method (1) was employed. The biological results were not very satisfactory when 0·5 c.c. of spray fluid was substituted for 1 c.c. It is considered that the spray which amounted to 0·4 c.c. over the area of the dish (nearly twice as much as the atomiser in the original machine) is too great in amount. The large quantity of spray falling on the dish led to a greater percentage kill for the same concentration of poison than was obtained

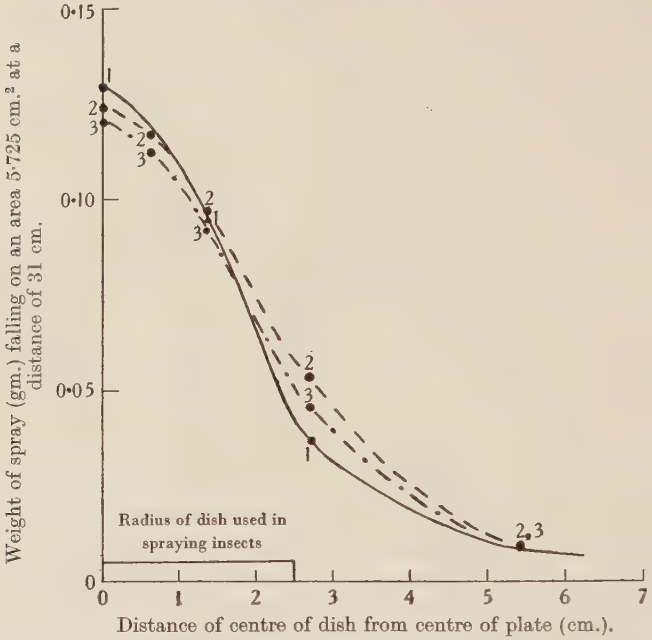

Fig. 2. Distribution of spray. Atomiser No. 1. Valve fully open.

●^{1}——●^{1} Water. ●^{2} — — ●^{2} 0·5 % saponin solution.
●^{3} —·— ●^{3} 0·5 % saponin + 5 % by vol. of alcohol.

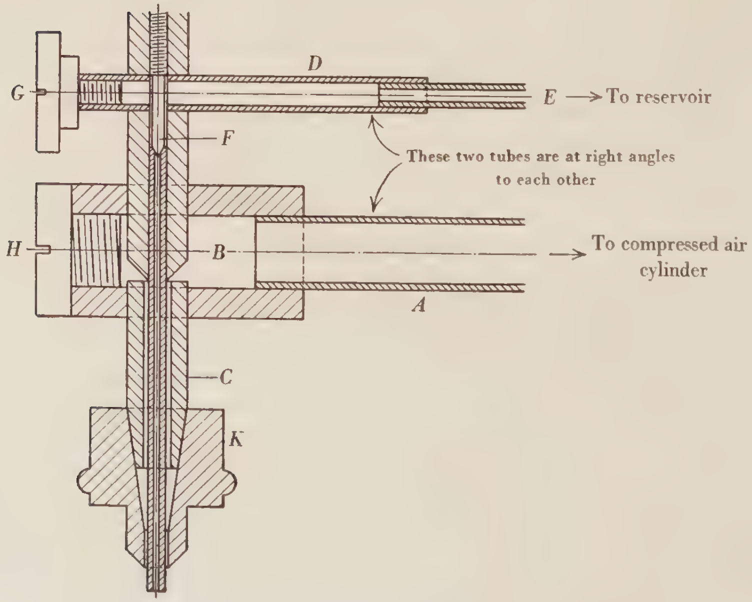
D
G
E ⟶ To reservoir
F
These two tubes are at right angles to each other
H
B
To compressed air cylinder
A
C
K

Fig. 3. Vertical section through atomiser No. 2 (twice actual size).

when the original atomiser was used. In view of these facts attempts were made to improve the evenness of the distribution over the surface of the dish.

ATOMISER NO. 2.

Mr F. W. Pellant designed another atomiser, which could not only be replicated more easily than the one described above, but gave a distribution of spray comparable with the original form. Its design is shown in Text-fig. 3.

The design of atomiser No. 2 differs from that of No. 1 in one important particular. The brass tube *C* tapers externally to take and hold by friction a spray cap *K*[1]. Internally this cap has a conically cut bore, and the air emerges from it much nearer to the orifice of the tube conducting the spray fluid; this leads to a greater scatter of the spray droplets. The other details of the atomiser are much the same as in the first model, *e.g.* the screw caps *G* and *H* can be detached, if it is found necessary, to clear the tubes *A* and *D*, either by blowing air down the tubes or by the insertion of a thin wire.

DISTRIBUTION OF SPRAY BY ATOMISER NO. 2.

The amount of spray delivered on to the surface of a dish 5·2 cm. external diameter (= 21·24 sq. cm. in area) placed at two different depths from the orifice of the atomiser, when 1 c.c. is sprayed, and when the needle valve is one turn, one half-turn or one-quarter turn open is shown in Table III.

Table III.

Weight of spray delivered on to an area of 21·24 *cm.*^{2}*
(5·2 *cm. diameter*) *by atomiser No.* 2, 1 *c.c. sprayed.*

Spray fluid: 0·5 per cent. saponin solution containing 5 % by vol. of alcohol.

Depth of dish from atomiser cm.	Needle valve open	Weight of spray gm.	Ratio of weights of spray	Ratio $(31{\cdot}5)^2 : (35{\cdot}3)^2 = 0{\cdot}7962$
31·5	One full turn	0·2445 (1)	(1):(2) = 1·1283	—
	One-half turn	0·2167 (2)	(2):(3) = 1·0917	—
	One-quarter turn	0·1985 (3)	—	Ratio (4):(1) = 0·7935
35·3	One full turn	0·1940 (4)	(4):(5) = 1·1312	Ratio (5):(2) = 0·7914
	One-half turn	0·1715 (5)	(5):(6) = 1·0910	Ratio (6):(3) = 0·7919
	One-quarter turn	0·1572 (6)	—	—

* This area differs slightly from the area of dish in Table I as the weight falling upon the external area of dish was taken.

The data in Table III show that atomiser No. 2 delivers less into the dish at about the same depth than atomiser No. 1, the amount being very nearly the same with the valve fully open at a depth of 31·5 cm. as the original type of atomiser formerly gave at 35–6 cm., but rather less when the depth is increased to the latter figure (2). The amount delivered is nearly inversely proportional to the square of the depths of the dish from the orifice of the atomiser (column 5). There is, however, a slight discrepancy, which indicates that the evenness of scatter is slightly greater at the greater depth and that either the cone of spray is slightly curved outwards, or, more probably, that the concentration of the spray in the centre is more thoroughly broken up as the depth increases. Since two different glass jars were used in these tests, it is evident that the atomiser can be centred with considerable accuracy, if care be taken in the operation. Data obtained by a further investigation on the effect of depth upon the evenness of the spray are given below. The ratios of the weights of spray when the needle valve was open to different degrees are given in column 4, and it is shown that the ratios are the same for the two depths when comparisons are made between the valve fully open and half-open and quarter-open, that is, for both depths the same change in the degree of openness of the needle valve introduced proportionally an equivalent change in the degree of scatter.

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[1] It is of considerable importance to have the cap *K* concentrically disposed round the orifice.

THE EFFECT OF REDUCING THE OPENING OF THE NEEDLE VALVE ON THE DISTRIBUTION OF THE SPRAY.

The data in Table III indicate that the reduction from one full turn to one-half turn of the needle valve increases the degree of scatter, a further reduction to one-quarter still further increases it, but to a somewhat less extent. The effect upon the distribution of the spray was further studied with the circular plate ruled in concentric circles, by weighing the deposit on a small dish placed consecutively at greater distances from the centre in one direction. The data with the standard errors of the means of five determinations in each position, except the outermost one, are given in Table IV and plotted in Text-fig. 4.

The figures in Table IV confirm the deduction drawn from Table III that this atomiser delivers less in the centre of the cone of spray than atomiser No. 1, and that there is a much greater degree of scatter and thus that the distribution falls off less rapidly as the distance from the centre is increased. The degree of scatter is increased, as the needle valve is more nearly closed, and also as the pressure is increased from 15 to 24 lb. per sq. in. The proportional effects are shown by the ratios; thus, when the figures are taken for the weights of the deposits given with the needle valve open divided by those when half closed, although the deposit at the centre is 1·23 times greater with the open valve than with the half-open, it has become nearly unity at a distance from it of 2·4 cm. and much less (0·716) at 4·8 cm. A similar kind of relationship exists between the values for the half-open and the quarter-open position, but the figure for the ratio after being nearly unity at a distance from the centre of 2·7 cm. rises again at 5·4 cm. Although the standard errors show that the result is scarcely significant, it indicates that the valve in an almost closed position apparently gives rise to some loss as a particulate cloud which either escapes from the jar or deposits only very slowly. The weights of the deposits are plotted against the distance of the centre of the dish from the centre of the plate in Text-fig. 4.

Table IV.

The effect on the distribution of spray of different valve openings. Atomiser No. 2.

Spray fluid: solution of 0·5 per cent. saponin in water containing 5 per cent. by volume of alcohol. Area of dish 5·725 cm.². Distance from orifice 31 cm. Pressure 15 lb./sq. in. and 24 lb./sq. in.

	Weight of spray deposit (gm.) at a distance (cm.) of centre of dish from centre of plate				
Needle valve open	Centre	1·35	2·7	5·4	8·1
Pressure 15 lb./in.²					
One full turn (1)	0·0749 ± 0·001	0·0655 ± 0·0007	0·0435 ± 0·0013	0·0078 ± 0·0003	0·0014
One-half turn (2)	0·0610 ± 0·001	0·0559 ± 0·0013	0·0404 ± 0·0012	0·0109 ± 0·0011	0·0029
One-quarter turn (3)	0·0535 ± 0·0004	0·0519 ± 0·0004	0·0414 ± 0·0004	0·0089 ± 0·0004	0·0024
Pressure 24 lb./in.²					
Half turn (4)	0·0535 ± 0·0005	0·0469 ± 0·0012	0·0343 ± 0·0008	0·0117 ± 0·0004	0·0069
Ratio (1) : (2)	1·228	1·172	1·077	0·716	0·483
,, (2) : (3)	1·134	1·077	0·976	1·225	1·208
,, (2) : (4)	1·140	1·192	1·178	0·932	0·420

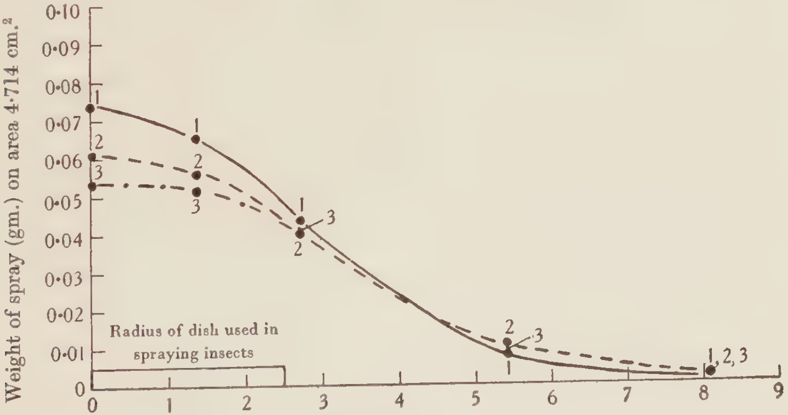


Fig. 4. The effect on the distribution of spray given by atomiser No. 2 by the degree of openness of the needle valve. ●¹———●¹ Valve open one full turn. ●² – – ●² Valve open one-half turn. ●³ – • – ●³ Valve open one-quarter turn.

THE EFFECT OF INCREASING THE DEPTH FROM THE ATOMISER ON THE DISTRIBUTION OF THE SPRAY.

An examination of Text-fig. 5 shows that the weight of the deposit of spray begins to fall off rather rapidly at a distance of about 2 cm. from the centre of the circle of spray. It was decided to study the effect of increasing the depth upon the distribution, particularly within the area covered by the dish used in actual spray trials upon insects. For this purpose the same plate was employed as in previous experiments, but a flat-bottomed glass dish of external diameter 1·1 cm. was used; the walls of the dish were relatively high (0·5 cm.) with respect to the diameter, and although this may have introduced some error it is probable that it is nearly constant for the several positions, and the dish has the advantage over a disc of partially inhibiting splashing out and in ease of handling. The dish was weighed before each spray trial in a weighing bottle, and after spraying, carefully lifted with forceps, dried on the bottom, reintroduced into the weighing bottle and weighed again. Two different glass jars were used for spraying, which was carried out at depths of 30·5 and 34·0 cm. with the valve open to different degrees. The distribution of the spray was determined in directions changing consecutively by 60°. When the complete data were obtained for the depth 30·5 cm. with the open valve it was found that centring was not perfect; the true centre was then found and the distances from it corrected. For subsequent spraying the glass plate inside the jar was moved so as to have its centre in the centre of the circle of spray deposit. The experimental error is relatively large owing to the small weight of deposit, and the data are only given as showing the order of the distribution at the two depths. There is no evidence to be drawn from the full data that, when correct centring had been obtained, the weight of deposit at different points equidistant from the centre differed by more than the experimental error. We have tabulated and plotted the data for directions to right and left of the centre when a view is taken of the apparatus at right angles to the one shown in C, Plate XXXVI. The results are shown in Table V and Text-fig. 5.

Table V and Text-fig. 5 show that increasing the distance of the dish from the orifice improves the even nature of the distribution of the spray over an area equal to that used in practice for spraying insects, and that it is comparable with that obtained with the original atomiser. One would expect that it would be further improved by lowering the dish to the depth at which it is intended to use it, namely 35·3 cm. Increasing

Table V.

Distribution of spray over area of dish used in spraying insects at different positions of needle valve and at different depths.

Spray fluid: 0·5 per cent. solution of saponin + 5 % by vol. of alcohol.

	Weight of spray (gm.) falling on dish 0·95 cm.² in area at a distance (cm.) of centre of dish from centre of plate						
	Left of centre			Centre	Right of centre		
	cm.	cm.	cm.	cm.	cm.	cm.	cm.
Depth 30·5 cm.*:	1·95	0·85	0·3	—	0·8	1·9	2·45
Valve one full turn	0·0118	0·0158	0·0173	—	0·0167	0·0116	0·0096
Depth 30·5 cm.:	—	2·2	1·1	—	1·1	2·2	—
Valve one-half turn	—	0·0110	0·0118	0·0147	0·0129	0·0110	—
Valve one-quarter turn	—	0·0097	0·0122	0·0135	0·0126	0·0114	—
Depth 34 cm.†:							
Valve one full turn	—	0·0098	0·0119	0·0128	0·0109	0·0113	—
Valve one-half turn	—	0·0075	0·0088	0·0109	0·0100	0·0093	—
Depth 34 cm.:							
Original atomiser	—	0·0096	0·0101	0·01145	0·0099	0·0077	—

* These data are corrected to the true centre.

† Six replications taken round the centre gave mean values: centre 0·0133 ± 0·0003 gm., at 1·1 cm. 0·0118 ± 0·0004 gm., and at 2·2 cm. 0·0108 ± 0·00035 gm. (valve open one full turn).

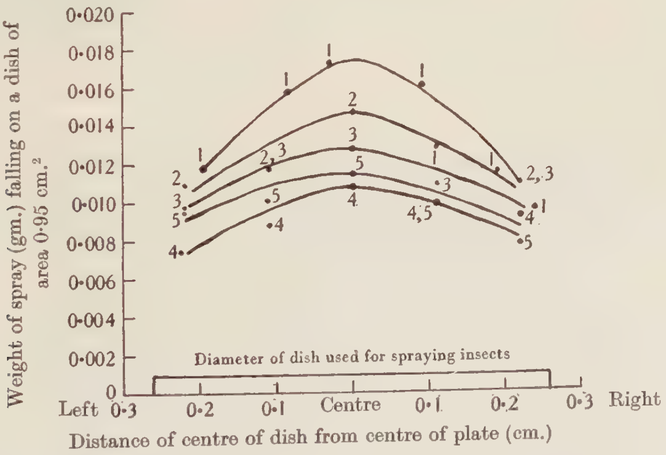


Fig. 5. Distribution of spray over an area 21·2 cm.² at two different depths.

1 ●——● 1 Valve one full turn. Depth 30·5 cm.
2 ●——● 2 Valve one-half turn. Depth 30·5 cm.
3 ●——● 3 Valve one full turn. Depth 34 cm.
4 ●——● 4 Valve one-half turn. Depth 34 cm.
5 ●——● 5 Original atomiser. Depth 34 cm.

the distance from the atomiser has a very similar effect to a partial closing of the needle valve. The ratios of the weights falling upon the dish at the two different levels do not agree so well with that of the inverse square of the distance as when the larger area was used (Table II), but this is to be expected, as over the smaller area the experimental error and the relative height of the walls of the dish are greater. The ratio of the squares of the depths from the orifice is $(30{\cdot}5)^2:(34)^2=0{\cdot}80$. With the valve open one turn, the ratio of the weights at the centre is approximately 0·74, and with the valve open one-half turn it is 0·73; with the valve in the latter position the ratios at 1·1 and 2·2 cm. from the centre are 0·76. As the weight falling on an area of 0·95 sq. cm. at the periphery of an area of a diameter 5·2 cm. (equal to that upon which the insects are sprayed) only differs from that at the centre by about 2·5–3 mg. at a depth of 34 cm. from the orifice, and will be less at 35·3 cm., it would appear that this atomiser could be safely relied upon for quantitative spraying, if distances of this order or more are used. An objection to using much greater depths arises from the possibility of eddy currents making quantitative delivery of the spray a matter of difficulty.

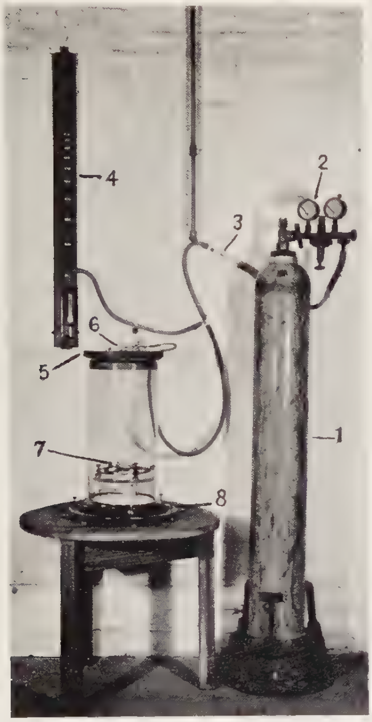
The atomiser can be made without the needle valve, and this may obviate any creeping of the spray fluid into the screw of the latter during actual spraying. A spraying chamber of square section, with a fixed lid and sliding door on one side for putting in the dish containing the insects, has also been constructed; the spray distribution is not affected by this form of apparatus, and although not so easy to wash out after use, it may have an advantage over a circular jar with movable lid in that there is less risk of inadvertently altering the position of the atomiser.

SUMMARY.

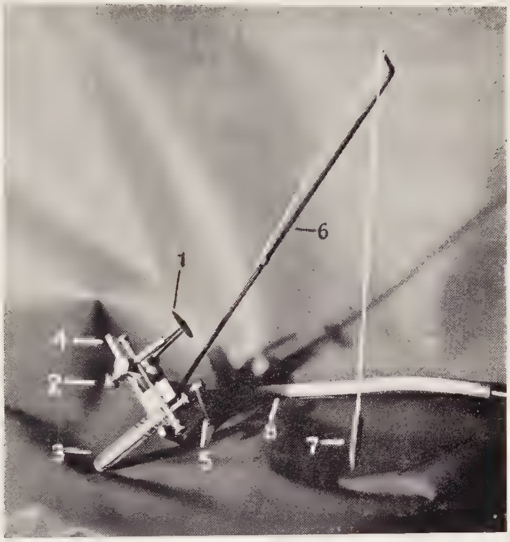
1. A description is given of the design of two atomisers for use in quantitative laboratory spraying for testing contact insecticides.

2. Tables and graphs are given showing the weights of spray delivered by each form upon known areas placed in different positions inside the spray jars, and an examination is made of the change in the distribution of the spray with the progressive closure of the orifice and increase in the distance from the orifice of the surface sprayed.

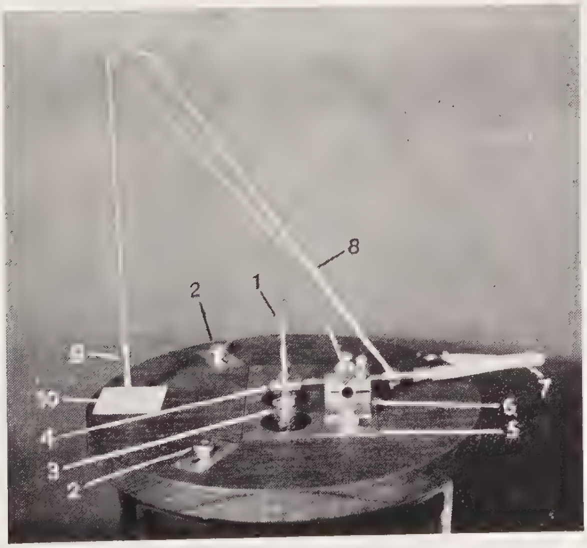
I am greatly indebted to Mr P. C. Bowes, formerly of this laboratory, for determining the distributions with atomiser No. 1, and especially to Mr F. W. Pellant, who designed and made the two atomisers and has supplied the drawings of them.



A. General view of apparatus.



B. Atomiser No. 2, clamp and plate detached from lid.


8
2
1
9
10
4
3
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7
6
5

C. Near view of lid with atomiser No. 2 in position.

TATTERSFIELD.—An Apparatus for Testing Contact Insecticides (pp. 691–703).

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(1) BLISS, C. I. (1934). The method of probits. *Science*, LXXIX, 38, 409.
(2) TATTERSFIELD, F. and MORRIS, H. M. (1924). *Bull. Ent. Res.* XIV, 223.

EXPLANATION OF PLATE XXXVI.

A. Complete apparatus, × about 1/20.
  1. Cylinder of compressed air.
  2. Pressure regulating valve.
  3. Air filter.
  4. Pressure gauge.
  5. Removable lid of jar.
  6. Atomiser.
  7. Internal platform (with dish) supported by glass plate on glass tripod.
  8. External levelling platform.

B. End view of atomiser No. 2, with brass plate and clamp removed from lid, × about 1/4.
  1. Milled head for adjusting needle valve.
  2. Orifice of atomiser No. 2 (showing cap).
  3. Glass reservoir with tube leading to atomiser.
  4. Brass plate removed from lid (atomiser is attached by clamp).
  5. Clamp.
  6. Gallows.
  7. Plummet.
  8. Tube leading to compressed air cylinder.

C. Near view of lid with atomiser in position, × about 1/4·6.
  1. Milled head for adjusting needle valve.
  2. Screws for locking adjustable blocks to give lid a firm fit on jar.
  3. Top of glass reservoir.
  4. Atomiser.
  5. Brass basal plate on which removable plate and clamp are held.
  6. Removable brass plate with clamp.
  7. India-rubber pressure tubing leading to compressed air cylinder.
  8. Gallows.
  9. Plummet.
  10. Squared paper.

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AN IMPROVED LABORATORY APPARATUS FOR FUMIGATION EXPERIMENTS

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(With Plate XXXVII and 7 Text-figures.)

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I. Introduction.

The apparatus described below has been devised and constructed in an attempt to minimise or eliminate some of the errors usually involved in the manipulation of apparatus employed in experimental work in fumigation. The chief difficulties encountered are concerned with:

(*a*) The loss of gas from the fumigation chamber occurring during the introduction and removal of the test animals.

(*b*) The provision of a simple mechanism for stirring the gas mixture so as to ensure a uniform concentration of lethal gas throughout the fumigation chamber.

(*c*) The determination of the gas concentration at any moment during the course of an experiment.

(*d*) The preparation of a gas mixture of a predetermined composition.

An examination of many of the appliances previously used in experimental fumigation work will disclose one or more of the above-mentioned difficulties in manipulation tending to introduce errors into the results of experiments. Much of the apparatus formerly employed could be used only with single gases or with air at atmospheric pressure, and in order to make experiments at reduced pressures or with mixtures of gases a different technique or special apparatus was needed. A second object in view was, therefore, to devise a single apparatus that should be adaptable for conducting experiments either with single gases or with gas mixtures at any desired pressure.

II. APPLIANCES USED BY OTHER INVESTIGATORS.

In general, the appliances hitherto designed for experimental fumigation work may be separated into two main groups. In the first group a glass flask, bell-jar, or other container serves as a fumigation chamber into which a definite quantity of fumigant is measured, usually with a pipette. In its most crude form the chamber is a glass flask closed by a rubber stopper which must be removed before the test insects can be put into or taken from the flask. These operations, naturally, cause a loss of fumigant, the extent of which cannot be ascertained. Apart from any convectional movement due to unequal heating of the chamber the only process tending to maintain a constant concentration of fumigant around the insects is that of diffusion. Moreover, precise knowledge of any change in concentration caused by absorption or leaks is unobtainable.

Owing to its simplicity many investigators have used this form of apparatus. In their researches on the toxicity of benzene derivatives and other organic compounds Moore (15, 16) and Moore and Graham (18) used flasks into which the substances were measured and allowed to evaporate from filter paper suspended inside. In a somewhat similar way Holt investigated the effects of various volatile liquid insecticides on cockroaches in a large, wide-mouthed, glass-stoppered bottle of about 1·25 litres capacity. Six-litre Erlenmeyer flasks were used by Lehman (12) in his experiments on the effect of fumigants on wireworms. After the flasks were filled with conditioned air, the insects, contained in copper-wire baskets, inserted and the desired quantity of liquid fumigant dropped in, the rubber stopper was put into place and sealed with paraffin wax. Chapman and Johnson (6), in experiments with chloropicrin as a fumigant for cereal products, made use of jars, each with a capacity of about 8 litres. A glass cover was put over the mouth of the jar after the insects and fumigant had been introduced.

Many of the tests, conducted by Roark and Cotton (22, 23) on various aliphatic compounds used as insecticides, were made in simple half-litre flasks. Cotton and Young (7) tested the efficiency of fumigants, when employed as mixtures with air and carbon dioxide, in a 6-litre flask, but their technique is inadequately described. Hamlin and Reed (10) made experiments to ascertain the effect of carbon disulphide on *Plodia* larvae in a 12-gallon bottle, the insects being held in cheese-cloth bags suspended inside. A jar of 500 ml. capacity sealed to a glass plate by vaseline was used by Boyce (1) for testing the resistance of *Sitophilus granarius* to hydrocyanic acid gas. The fumigant was blown into the jar by an atomiser inserted through a hole in the glass plate.

The carbon dioxide output of insects when subjected to the action of a lethal gas was investigated by Schafer (24), who made use of cork-stoppered flasks in which the insects were suspended in a small cage. In other experiments, a wide-mouthed jar having a two-way stopcock at the top was utilised as a respiration chamber. The bottom was closed by a large rubber cork which had to be removed to introduce the insects. The cork was then replaced and mercury run into the jar from a raised aspirator bottle. As the air was displaced through the stopcock the insect was carried to the top of the jar on the surface of the mercury. Then, by lowering the aspirator bottle, the required gas mixture was drawn into the respiration chamber. Samples of gas for analysis were taken through the stopcock at the beginning and at the end of each experiment.

In 1927, Strand (25), when making comparisons of the toxicity to insects of chloropicrin, carbon disulphide, and carbon tetrachloride, constructed a fumigation chamber from a large bell-jar standing on a ground-glass plate. A small bell-jar inside the large one was provided to protect the insects from the action of the fumigant before the experiment actually began. After the large bell-jar had been placed in position on the glass plate and sealed with plasticine, the cork closing the glass vial which held the fumigant was released by an attached string and the fumigant spilled over crumpled filter paper. A few minutes after the last trace of liquid was seen to evaporate the small bell-jar was lifted. There was no means of sampling the gas mixture and no stirring mechanism for keeping an even concentration in the jar. Moreover, there was a possibility of a loss of gas, through an opening in the side of the jar through which the small bags, containing the insects, were extracted at definite intervals.

The same experimenter, in 1930 (26), measured the toxicity of insect fumigants in a 6·4-litre Erlenmeyer flask closed by a rubber stopper. The insects, *Tribolium confusum* Duv., were suspended inside the flask in small bags and the fumigant dropped in from a burette. Strand's method was, in general, adopted by Lindgren and Shepard (13), who ascertained the influence of humidity on the effectiveness of certain fumigants against the eggs and adults of *T. confusum*; but certain modifications were introduced, especially an improved flask designed by Strand. A ground-glass stopper was substituted for the rubber cork and a long and a short tube sealed to it, the former reaching to near the bottom of the flask and the latter opening into the flask near the top. By this means increased ventilation was secured when the flask was being flushed by a stream of conditioned air. Fumigants were measured volumetrically by pipettes and dropped into the flask.

In 1911, McClintock, Hamilton and Lowe (14), in their experiments on fumigants used as insecticides, employed as a fumigation chamber a large bell-jar of 15·55 litres capacity, sealed at the bottom and provided at the top with a cork carrying two tubes. One tube was connected to a manometer and the other to a suction pump. A partial vacuum of about 68 cm. was drawn, after which the fumigant, volatilised by heat outside the jar, was allowed to flow in. When the desired quantity of fumigant had been introduced, the gas pressure in the jar was restored to atmospheric pressure with air. By means of the manometer definite mixtures of gases could be made.

Jones (11) describes an apparatus somewhat similar in principle. A 5-litre Pyrex glass flask was closed by a cover which fitted to the neck by ground-glass flanges. To the cover two tubes were sealed, one of which reached nearly to the bottom of the flask and could be connected through a ground-glass connection with a manometer and a small flask which held the necessary fumigant. The other tube opened into the flask near its centre and carried, at its lower end, a small cage of bolting cloth into which the experimental insects could be dropped from the other end. A glass stopper closed the exit. By noting the partial pressure exerted by the fumigant, which evaporated from the small flask into the evacuated fumigation chamber, the desired concentration of gas was obtained. Definite quantities of other gases could be admitted as required. Fumigants with a high boiling-point were measured into the apparatus with graduated pipettes.

A different appliance was employed by Gray and Kirkpatrick (9). A cubical box of 27 c. ft. capacity, constructed of aluminium alloy welded at the seams, served as a fumigation chamber. It was open at the bottom and the free edges dipped into a trough filled with "white oil" which acted as a seal. Presumably, the scale insects upon which the experiments were made had to be placed in the chamber by lifting the box out of the trough. Samples of gas were withdrawn for analysis before and after each exposure and the fall in pressure, caused by the withdrawal of gas, was compensated by the expansion of a rubberised, gas-proof, fabric bag attached to the inside of the chamber but opening to the exterior. Moore (17) improved this apparatus by substituting mercury for the oil seal and by installing a fan to ensure a uniform distribution of gas. Dosages of hydrocyanic acid gas were measured into a little chamber, the entire quantity being dropped on to a heated coil to ensure rapid volatilisation.

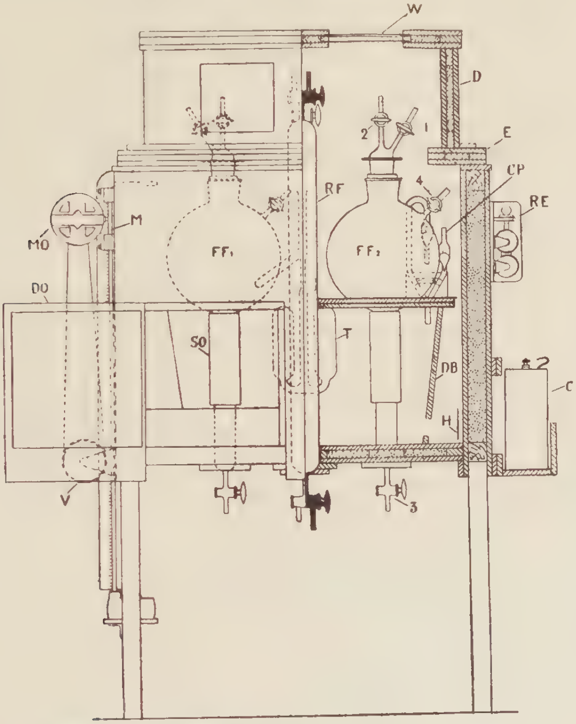
Camp and Wilmott (3, 4, 5) used a more or less similar method in their fumigation research in Florida on the concentration of hydrocyanic acid gas necessary to kill the black scale (*Aleurocanthus woglumi* Ashby), and the green scale (*Coccus viridis* Green). The insects were confined in a short glass tube inserted in the suction line to the gas-sampling bulbs. To compensate the loss of gas entailed by the sampling and to allow for changes of barometric pressure, the box was lifted or lowered sufficiently to restore the internal pressure to atmospheric as indicated by an attached manometer. The designers state that in a 10-hour run, with a blank experiment, the loss of fumigant from the chamber amounted to 2·5 per cent. per hour.

In the second group of experimental fumigation appliances is the apparatus which appears to have been used first by Niefert and Garrison (19). A gas concentration of a desired strength was established in a fumigation chamber by maintaining a continuous flow of gas through it. The gas mixture was obtained by combining a current of conditioned air with a current of air carrying the lethal agent. The flow of each current was regulated by adjusting a flowmeter which was inserted in each circuit. This is known as the flowmeter method and the technique involved is a great improvement on that of the former method. If one can ensure a constant concentration of gas flowing through the fumigation chamber at a constant rate the usual objections made against experimental fumigation appliances are not valid. There is a constant movement of gas round the insects, samples of gas may be taken for analysis at any time in the course of an experiment and the manipulation of the insects offers no difficulty. The method has been employed by Niefert and others (20), Brinley and Baker (2), Richardson and Haas (21) and Cupples (8).

This flowmeter apparatus as described and used by these workers does not, however, lend itself readily to experimental work when mixtures of gases are employed and does not allow of work under reduced pressures.

III. DESCRIPTION OF THE APPARATUS USED BY THE AUTHOR.

The apparatus comprises a fumigation flask and a control flask housed in a constant temperature cabinet as shown in Text-figs. 1 and 2.



Text-fig. 1. Front elevation of fumigation apparatus. The right half is a section taken through the reserve gas flask, parallel to the front of the cabinet. In order to avoid confusion the connections of the flasks with the accessory apparatus are not shown; they are given in Text-fig. 3.

(1) *The constant temperature cabinet.*

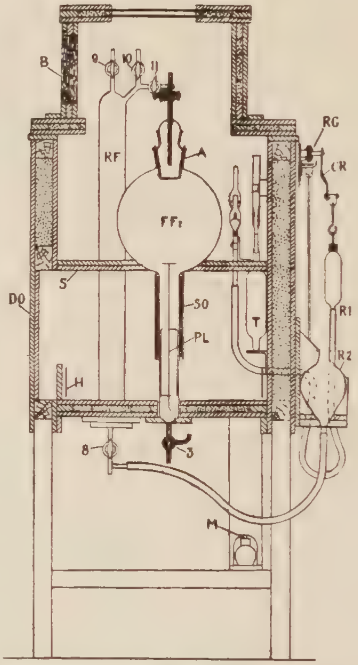
(Plate XXXVII, Text-figs. 1 and 2.)

The lower and larger portion containing the flasks consists of a frame constructed of batten, $1\frac{1}{2} \times 1\frac{1}{2}$ in. in section, over which matchboarding $\frac{1}{2} \times 6$ in. is fastened, the hollow sides and bottom so formed being filled with slag wool. It rests on four legs about 18 in. from the ground. The top (*E*) of this section, which can be lifted off when necessary, supports the upper and smaller part (*D*) of the cabinet covering the tops of the flasks and the accessory apparatus. The sides and roof of this portion are also hollow and insulated with slag wool.

To facilitate the manipulation of the apparatus during an experiment two small double-glass windows (*W*), one over each flask, have been fitted into the top of the cabinet, through which the apparatus can be seen. Internal illumination is provided for by two small 4·5-volt electric lamps fed from a battery of cells (*C*) carried on a shelf attached to the right-hand side of the cabinet.

In the front of the upper portion (*D*) are two square holes (*B*) each provided with two pieces of ⅝ in. felting, so fastened that by lifting outwards the loose half of the inner piece a hole is formed through which a hand may be inserted for the manipulation of the stopcocks connected with the apparatus. Two small doors (*DO*) are fitted in the lower part of the cabinet and give access to solenoids, thermostats and heater.

The cabinet is heated by a 100-watt woven asbestos heating mat (*H*) fixed near the floor, the side portions being covered by an obliquely fitted deflector board (*DB*) which causes the air, heated by the mat, to flow upwards along the sides of the cabinet and across the top of the flasks. Air returns to the heater through large holes in the base of the deflector board. The heating is controlled by a mercury-toluol thermostat (*T*) fixed near the back of the cabinet and connected to the heater through a relay (*RE*) and a mercury switch. An overload thermostat, adjusted to work at a temperature slightly above that at which the cabinet is to be maintained, cuts off the heating current in the event of the main thermostat or thermostat circuit being at fault. This overload thermostat, its relay and mercury switch are not shown in the figures.



Text-fig. 2. Section of apparatus taken through the centre of the control flask. All connections between flasks and accessory apparatus are omitted.

(2) *The fumigation flask* (FF_1).

This is a large glass globe, 20 cm. in diameter, from the bottom of which projects a neck 4 cm. in diameter and about 28 cm. long. The neck terminates in a three-way stopcock (3) through which samples of the gas mixture from inside the flask may be taken. In the top of the flask, opposite the neck, is a wide mouth fitted with a small animal chamber (A), a detailed sketch of which is given in Text-fig. 4. It takes the form of a hollow stopcock, the barrel (K) being cemented into the mouth (N) of the flask so that its lower portion projects inside the flask to a distance of about 2 cm. This lower part carries two holes (O), each 1 cm. in diameter, opposite one another. The hollow plug (L) fits into the barrel and can be turned by the aid of two lugs (H). On the base (P) of the plug are two other holes, which in one position lie over the corresponding holes in the barrel, and afford communication between the animal chamber and the flask. Thus, by rotating the plug (L), the animal chamber can be closed off from, or placed into connection with, the interior of the flask.

The animal chamber, the volume of which when closed is about 70 ml., carries a stopper (Y) bearing two tubes each provided with a stopcock (1 and 2). One tube reaches nearly to the bottom of the chamber and the other leads off from the top. This arrangement allows the animal chamber to be flushed by a stream of gas mixture forced from the reserve flask. A short tube carrying a three-way stopcock (4) is sealed into the side of the flask. A solenoid (SO), actuating a light nickel-plated steel plunger (PL) in the flask, is fitted round the neck, as shown in Fig. 2.

(3) *The control flask* (FF_2).

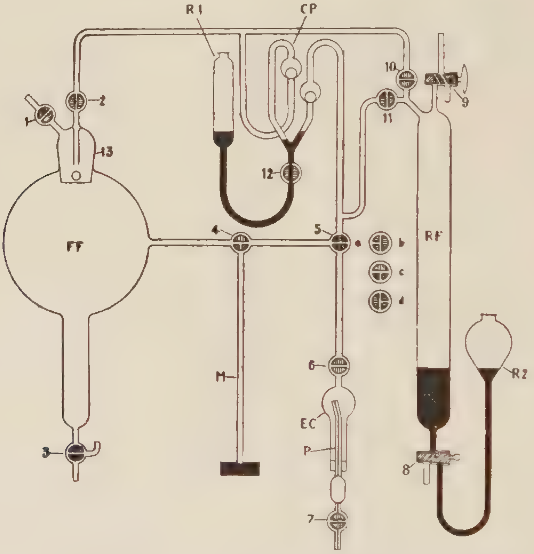
This flask is similar to the fumigation flask except that stopcock 4 is a single-way cock.

(4) *The reserve gas flask* (RF).

The reserve gas flask is a glass cylinder 5 cm. in diameter and 60 cm. long. At the upper end is a three-way stopcock (9) through which the flask and the rest of the apparatus may be evacuated. A Y-piece carrying two single-way stopcocks (10 and 11) is also fused on here. At the lower end of the vessel is another three-way stopcock, which provides means for taking a gas sample as well as for making connection with a mercury reservoir (R_2) situated outside the cabinet. This reservoir rests in a wooden seat which can be raised and lowered by means of a chain fixed to the back of the cabinet. If the reservoir is raised and stopcock 8 opened mercury flows into the reserve gas flask and displaces some of the contained gas mixture through one of the upper stopcocks 10 or 11.

(5) *Other accessories.*

Two small glass circulating pumps (CP), actuated by mercury, are fitted inside the cabinet, the left-hand one being connected to the fumigation flask and the other to the control flask. In Text-figs. 1 and 2 only the right-hand one is shown. Mercury is made to rise and fall in the leg and lower portions of the arms of the pumps by the raising and lowering of a mercury reservoir R_1 outside the cabinet. As the reservoir is lowered air flows into the pump from the animal chamber (Text-fig. 3) through the left-hand valve, the right-hand valve being held to its seating by the pressure of the air in the fumigation flask. The raising of the reservoir compresses the air in the left-hand limb of the pump and keeps the left-hand valve on its seating. Simultaneously the pressure developed in the right-hand limb causes the corresponding valve to lift. These reciprocating movements force air to flow from the left limb to the right and, thereby, from the animal chamber, through the pump, to the main body of the fumigation flask. The connections between this flask and its circulating pump are shown in Text-fig. 3. The other pump

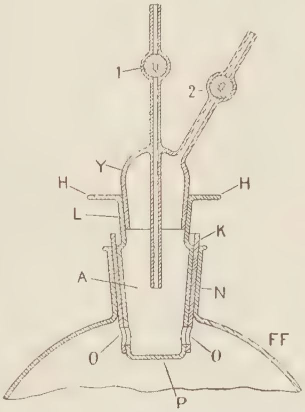


Text-fig. 3. Diagrammatic sketch to show the interconnections of the fumigation flask with the accessory apparatus.

A, animal chamber; *B*, felt-covered aperture; *C*, battery of cells for internal lighting; *CP*, circulating pump; *CR*, connecting rod; *D*, upper portion of cabinet; *DB*, deflector board; *DO*, door; *E*, support for *D*; *EC*, evaporating chamber; *FF* and FF_1, fumigation flask; FF_2, control flask; *H*, heating unit; *M*, manometer; *MO*, motor for working circulating pumps and solenoids; *P*, pipette; *PL*, plunger or stirrer; *PU*, vacuum pump; R_1 and R_2, mercury reservoirs; *RE*, relay to thermostat; *RF*, reserve gas flask; *RG*, upper reduction gear; *S*, shelf to support flasks; *SO*, solenoid; *T*, thermostat; *V*, pulley and shaft driven by motor *MO*; W, window; 1–12, stopcocks; 13, animal chamber.

is connected to the control flask so as to cause a circulation of air similar to that taking place in the fumigation flask.

The motion of the mercury reservoir R_1 is produced by the rotation of the crank *CR*. This is turned by a small electric motor (*MO*) driving a pulley and shaft (*V*), whose motion is transmitted to the crank through two worm and worm-wheel reduction gears. The crank is made to revolve about twice a minute and the volume of air passing through the pumps, which are both connected to reservoir R_2, can be varied from about 6 to 12 ml. by adjusting the quantity of mercury in the reservoir. If the pumps are set to deliver 10 ml. of air during each revolution of the crank about 1200 ml. pass through the animal chambers every hour. Since the cross-sectional area of the animal chamber is about 10 sq. cm., the average linear velocity of the current of air over the insects is about 1·2 metres per hour. The drying effect of this slowly moving air on the experimental insects may be considerable, but the speed of the air current in the control animal chamber is also about 1·2 metres per hour, since the quantities of air delivered per hour by each pump are the same. A stopcock (12) is inserted in the connection between the reservoir and the pumps and is closed whilst the apparatus is being exhausted.



Text-fig. 4. Sketch showing details of animal chamber of the fumigation flask. *A*, animal chamber; *FF*, fumigation flask; *H*, lugs for turning *L*; *K* and *L*, barrel and plug of animal chamber; *N*, neck of flask; *O*, holes in barrel and plug; *P*, bottom of plug *L*; *Y*, stopper to animal chamber; 1 and 2, stopcocks.

A manometer (*M*), formed from glass tubing of 2 mm. bore, dips into a small mercury chamber and is connected to the fumigation flask as shown in Text-fig. 3. It serves to register the pressure of the atmosphere within the apparatus and also as a gauge when making up gas mixtures of a definite composition. It is fixed to the left-hand outside wall of the cabinet and is provided with a millimetre scale.

The stirrer or plunger (*PL*) is a short cylinder of thin sheet iron loosely fitting the inside of the long necks of the flasks. The ends are not quite closed and carry through their centres a thin wire, the upper end bearing a circular plate of metal and the lower end being bent into a hook which acts as a spring buffer when the plunger drops. The whole of the plunger is nickel-plated and there is one plunger in each of the experimental and control flasks.

Small graduated pipettes, capable of delivering from 0·0005 to 0·2 ml. of liquid, are used for introducing the fumigant into the flask. They are closed at one end by a stopcock (7), the other end being inserted into a small evaporating chamber (*EC*) through a rubber cork. The exit tube of the evaporating chamber carries a stopcock (6). A definite quantity of fumigant can be taken up by one of these pipettes and the whole of it transferred to the fumigation flask. When working with an extremely poisonous substance such as hydrocyanic acid this form of pipette is very convenient, as it can be loaded in the open air and brought into the laboratory without any danger of spilling or loss. The evaporating chamber is U-shaped, so that it can be immersed in a beaker of water placed on the floor of the constant temperature cabinet. This is necessary because the chamber and pipette are cooled down whilst loading, and if warm moist air from the conditioning apparatus were then passed through, moisture would be condensed and the humidity of the air in the fumigation flask would be reduced.

The air and gases passed into the flasks are conditioned by being slowly bubbled through sixteen large gas-washing bottles of 250 ml. capacity and each containing about 30 ml. of caustic soda solution or sulphuric acid solution as necessary. The concentration of the solution is adjusted to condition the air to the desired humidity, the air afterwards passing through a tall bottle packed with glass-wool. The whole of this conditioning apparatus is housed in a separate incubator maintained at the same temperature as the fumigation cabinet, the connecting tube between the two cabinets being well insulated. When two or more gases are being used, a separate series of gas-washing bottles must be used for conditioning each gas. An "Edney" direct reading hygrometer housed in a small glass container serves to register the relative humidity of the air which passes through the container prior to its entering the flasks.

A Gaede, single-stage, rotary, oil-sealed, vacuum pump (*PU*), driven by a 1/6 H.P. electric motor, is installed for evacuating the flasks. It is connected by pressure tubing to stopcock 9.

(6) *Interconnections of the apparatus.*

Text-fig. 3 is a diagrammatic sketch showing the connections of the fumigation flask to its accessories, all the stopcocks being in their correct positions whilst an experiment, such as described immediately below, is in progress. The connections are formed, as far as possible, of glass tubing with butt joints covered with rubber pressure tubing. In order that stopper *Y* may be taken out of the animal chamber a piece of rubber tubing about 6 in. long connects stopcock 2 to the glass tubing from the circulating pump. The positions of the butt joints are shown in the figure.

IV. PROCEDURE FOR AN EXPERIMENT WITH HYDROCYANIC ACID GAS-AIR MIXTURE AT ATMOSPHERIC PRESSURE.

In the following account, the periods of time placed in brackets are those necessary to carry out the operations described. If humidity is to be one of the controlled factors in the experiment the control flask must be filled with air at the same relative humidity as that in the fumigation flask.

(1) Stopcocks 1, 2 and 4 of the control flask are closed, 13 and 3 are opened and the latter connected to the vacuum pump. The flask is exhausted (5 min.). Cock 3 is closed and then connected to the conditioning plant. Cock 3 is opened and conditioned air flows into the flask slowly until it is full (30 min.). Cock 13 is closed.

(2) The required quantity of hydrocyanic acid is taken up in a pipette by capillary action and the pipette placed in the evaporating chamber. Cocks 6 and 7 are closed (2 min.). This operation is conducted in the open air whilst the experimenter is wearing a gas mask. It must be noted that it is necessary to take up sufficient fumigant to give, throughout the whole of the apparatus shown in Text-fig. 3—except the space beyond cock 5 and that inside the animal chamber—a concentration of gas equal to the final concentration required in the fumigation flask. Cock 6 is connected to 5, and 7 is attached to the supply of conditioned air.

(3) If the reserve flask contains mercury it is emptied by lowering reservoir R_2. Cock 8 is closed. The animal chamber is shut off from the fumigation flask by closing cock 13. Cocks 1, 10 and 11 are opened, 2, 3 and 12 closed, 5 is put into position "*b*" and 4 into position "*C*."

(4) The reserve gas flask is connected to the exhaust pump through cock 9, which is opened, and the whole apparatus, so far as the open cocks will allow, is evacuated (5 min.). Cock 5 is turned to position "*C*."

(5) Cock 6 is gradually opened. The fumigant from the pipette is volatilised under the reduced pressure and is drawn into the fumigation flask with conditioned air which bubbles slowly through the conditioning apparatus on slightly opening cock 7. After the manometer registers atmospheric pressure (30 min.) cock 6 is closed and the plunger made to rise and fall several times by tipping with the finger the mercury switch in the solenoid circuit. When the mixture is thoroughly stirred cock 5 is

turned to position "*a*," which allows the gas mixture to flow from the fumigation flask into all other parts of the apparatus which are exhausted of air (3 min.).

(6) The evaporating chamber is disconnected from cock 5 which is then placed directly into communication with the conditioning plant. Cock 5 is opened slightly towards position "*b*," when conditioned air flows in slowly until the manometer again registers atmospheric pressure (10 min.). Cock 5 is again turned to position "*a*," cock 11 is closed and the mixture stirred by actuating the plunger.

(7) The experimental insects, contained in a small muslin bag, are placed in the animal chamber and the stopper *Y* replaced. Cocks 2 and 8 are opened and reservoir R_2 raised so as to cause a quantity of gas mixture, equal to three or four times the volume of the animal chamber, to flow from the reserve flask through the chamber by way of cocks 10, 2 and 1. This action flushes out most of the air from the animal chamber and replaces it with a mixture nearly similar in composition to that in the fumigation chamber (1 min.). An absorption tube containing sodium hydroxide is attached to cock 1 in order that the expelled hydrocyanic acid gas shall not escape into the room.

(8) Cocks 1, 8 and 10 are closed and 13 is opened, so that the animal chamber and fumigation flask are in connection with each other. Cock 12 is opened and the motor (*MO*) started immediately. The experiment is noted as beginning with the starting of this motor.

(9) Whilst the fumigation flask is filling up with air, as noted in operation (5), the control insects, contained in a muslin bag, are put into the animal chamber of the control flask by quickly lifting and replacing stopper *Y*. Immediately operation (8) is completed, cocks 2, 4 and 13 of the control flask are opened. This allows a circulation of air through the animal chamber and flask similar to that in the fumigation flask. The change in relative humidity of the air in the animal chamber which takes place while the insects are being inserted is not sufficient to cause an appreciable error in the air passing over the control insects. A gas sample is then taken from the fumigation flask through cock 3.

(10) On completion of the experiment:

(i) The motor is stopped so as to leave the reservoir R_1 in its lowest position. Cocks 2 and 13 of the fumigation flask and cock 12 are closed. The insects are extracted from the animal chamber.

(ii) Cocks 2 and 13 in the control flask are closed and the control insects taken out.

(iii) A gas sample is taken from the fumigation flask.

(iv) The tube from the conditioning plant is disconnected from cock 5; cocks 1, 2, 10, 11 and 8 are opened and reservoir R_2 lowered so as to empty the mercury from the reserve flask. Cocks 1 and 8 are closed and the apparatus exhausted and refilled with air twice so as to wash out all residual fumigant before proceeding to another experiment.

V. PROCEDURE FOR AN EXPERIMENT WITH HYDROCYANIC ACID GAS IN AN ATMOSPHERE COMPOSED OF 80 PER CENT. AIR AND 20 PER CENT. CARBON DIOXIDE AT ATMOSPHERIC PRESSURE.

When working with a mixture of two gases in addition to a lethal substance, the gas which is in the lesser quantity should be admitted to the flask first, together with the fumigant. When the partial pressure attained, as measured by the fall of the mercury in the manometer, is equal to that exerted by the gas in the required mixture, due allowance being made for the vapour pressure of the fumigant, the remaining gas is passed in until atmospheric pressure is restored in the apparatus.

However, the gas must first be passed into the fumigation flask (4·21 litres capacity) and afterwards be permitted to expand into the remainder of the exhausted apparatus (1·115 litres capacity). This means that the partial pressure exerted by the gas whilst it occupies only the fumigation flask must be higher than that exerted by the same quantity of gas after it has expanded into the reserve flask and the interconnections. The relation between the magnitudes of these two partial pressures depends upon the ratio between the volume of the fumigation flask and that of the whole apparatus, and is shown in Text-fig. 5. An ordinate on the figure represents the pressure exerted by a quantity of gas occupying the fumigation flask together with the space between the flask and cocks 4, 5 and 6, but excluding the animal chamber. The corresponding abscissa represents the pressure due to the same quantity of gas after it has expanded and filled the rest of the apparatus excluding the animal chamber. If the latter quantity is known the former may be obtained from the figure.

In the experiment under consideration it is assumed that the concentration of hydrocyanic acid gas required is 10 mg. per litre. The quantity of fumigant which must be introduced is, then, $(4{\cdot}21+1{\cdot}115)\times 10=53{\cdot}25$ mg. From Text-fig. 6 it is seen that this quantity, when passed into the fumigation flask, will exert a vapour pressure of 8·6 mm. when the barometric pressure is 760 mm. The partial pressure due to the carbon dioxide which is admitted with the fumigant must therefore be $20\times(760-8{\cdot}6)\div 100=150{\cdot}3$ mm. From Text-fig. 5

it is found that this pressure is equivalent to one of 187·0 mm. in the fumigation flask.

The procedure adopted is therefore as follows:

Operations (1) and (2) are carried out as described above, except that cock 7 is connected to the supply of conditioned carbon dioxide (passed through sulphuric acid solution) instead of to the air-conditioning bottles.

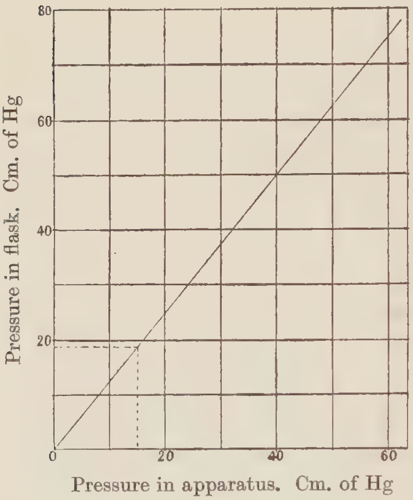


Fig. 5.

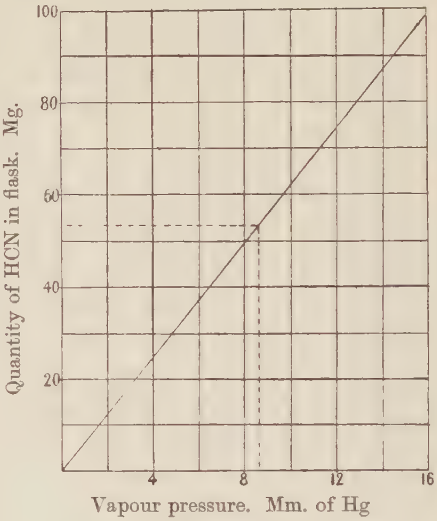


Fig. 6.

Text-fig. 5. Showing relation between pressure in fumigation flask and in whole apparatus.
Text-fig. 6. Vapour pressure of HCN in fumigation flask.

Operations (3), (4) and (5) are then completed. On opening cock 7 in operation (5) the hydrocyanic acid is drawn into the flask with the carbon dioxide. When the manometer registers a pressure of 195·6 mm., *i.e.* 187 + 8·6 mm., cock 6 is closed and the mixture in the flask is thoroughly stirred. Cock 5 is then opened to position "*a*," so that the gas mixture expands and fills the remainder of the apparatus. The manometer should now register 156·5 mm.

The evaporating chamber and pipette are disconnected from cock 5 which is then attached to the air-conditioning plant. Cock 6 is opened and 5 turned slightly towards position "*b*," which allows air to flow in slowly until atmospheric pressure is restored in the apparatus. Cock 5 is turned back to position "*a*," 11 is closed and the gas mixture thoroughly stirred.

The remaining operations are similar to (8), (9) and (10) in section IV. On the completion of the experiment the manipulation of the apparatus is the same as described for the preceding experiment.

VI. MISCELLANEOUS NOTES.

(1) *Heating of the cabinet.*

The fluctuations of temperature in various parts of the cabinet and fumigation flasks have been ascertained from observations made over a number of days. Whilst the cabinet was being run at 25° C., the temperature inside the animal chambers and in the centre of the flasks did not vary more than ±0·15° C.

(2) *Loss of hydrocyanic acid gas from the apparatus during an experiment of 5 hours' duration.*

During an experiment similar to that described above, there is a loss of gas due to absorption by the rubber tubing employed for making the connections in the apparatus, by stopper grease, by the tissues of the insects and by the fabric of the muslin bag containing them. This loss of gas has been ascertained in each of nineteen experiments on *Sitophilus oryzae* and *S. granarius*, and the details are given in Table I.

Table I.

Loss of hydrocyanic acid gas from the apparatus.

Experiment	Initial concentration of hydrocyanic acid gas mg. per litre	Loss of gas in experiment of 5 hours' duration mg. per litre	Percentage loss of gas in 5 hours
1	0·889	0·014	1·57
2	1·930	0·084	4·35
3	2·797	0·034	1·22
4	4·105	0·150	3·65
5	6·108	0·136	2·23
6	6·625	0·070	1·06
7	7·353	0·146	1·98
8	7·619	0·118	1·55
9	8·421	0·183	2·17
10	9·589	0·179	1·87
11	9·662	0·124	1·28
12	10·680	0·180	1·68
13	11·180	0·290	2·59
14	12·715	0·370	2·91
15	13·260	0·420	3·16
16	16·125	0·470	2·91
17	18·430	0·500	2·71
18	19·070	0·460	2·41
19	20·020	0·690	3·44

The average loss of gas during each experiment in this series is 2·36 per cent., which is equal to 0·47 per cent. per hour. In the titrations made in Exps. 1–12 and 13–19, one drop of standard iodine solution was equivalent to about 0·015 mg./litre and 0·030 mg./litre respectively.

(3) *Effect of mercury vapour from the pumps upon the insects in the flasks.*

It was thought possible that traces of mercury vapour collected by the gas mixture while passing through the circulating pumps might have had a deleterious effect upon the insects in the flasks. As a precaution a small roll of gold foil was pushed into one of the tubes in the gas circuit of each flask. A number of experiments were then made in each of which a batch of insects was placed in the control flask and a similar batch in the incubator which housed the humidifying plant. During the experiments the temperature was maintained at 25° C. and the relative humidity about 45 per cent. in the flask and 45–55 per cent. in the incubator. Each experiment lasted 5 hours, and Table II shows the results.

Table II.

Effect of mercury on the insects.

Insect	No. of experiments	No. of insects in control flask	Percentage dead	No. of insects in incubator	Percentage dead
Tribolium castaneum	11	382	0·5	325	3·1
Sitophilus oryzae	18	953	1·8	948	2·1
S. granarius	14	734	3·54	769	3·4

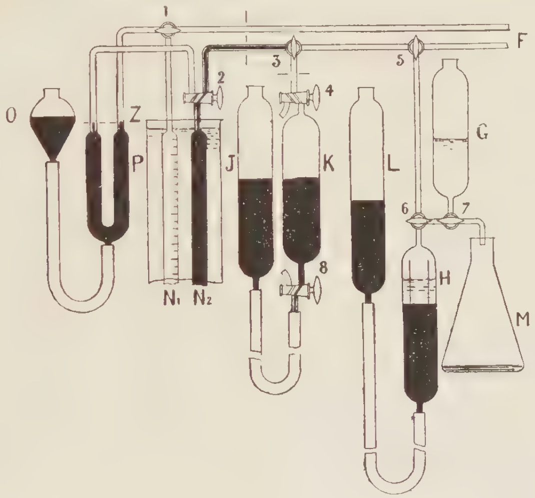
These results show that the quantity of mercury vapour present in the air circulating in the flasks is not sufficient to be detrimental to the insects under the conditions prevailing during the tests.

(4) *Vapour pressure due to hydrocyanic acid gas in the flask.*

A series of experiments were made with various quantities of hydrocyanic acid gas to ascertain the partial pressure exerted in the fumigation flask by the gas. The relation existing between the quantity introduced and its vapour pressure at 25° C. is shown in Text-fig. 6.

(5) *Determination of hydrocyanic acid gas in the fumigation chamber.*

In the experiments with hydrocyanic acid gas the concentration of the gas mixture has been ascertained by absorbing the vapour, from approximately 100 ml. of gas mixture, in caustic soda and titrating with standard iodine solution. Text-fig. 7 shows the absorption tube *H*, which forms an addition to a Haldane's gas analysis apparatus not shown in the figure. The soda solution is contained in a reservoir *G* and passed, in aliquot parts, into *H*, into which the gas mixture from the sampling tube *K* is also admitted. By alternately raising and lowering the mercury reservoirs *J* and *L* and by suitable adjustment of the stopcocks, the gas is driven to and fro in *H* and *K* and the hydrocyanic acid absorbed in the soda solution in *H*. After absorption the soda solution is passed over into the flask *M* in which the titration with iodine is made.



Text-fig. 7. Sketch showing apparatus for HCN absorption.

F, junction for Haldane's gas analysis apparatus; *G*, reservoir for NaOH and water; *H*, HCN absorption tube; *J*, *L*, *O*, mercury reservoirs; *K*, gas sampling tube; *M*, flask for reception of NaCN after HCN absorption; N_1 and N_2, burettes; *P*, manometer; *Z*, zero line for manometer; 1, 3, 5, 6, 7, three-way stopcocks.

The volume of the gas sample is measured in burette N_2 by balancing it against a definite quantity of air in N_1, enclosed under known conditions. The balance is obtained by adjusting the levels of the mercury columns in the manometer *P* to the mark *Z*, when the volume of the air in N_2 can be read off at N.T.P. The mercury reservoir *O* has been added to facilitate the adjustment of the manometer.

(6) *Conclusion.*

From the preceding description of the apparatus and of the technique of the two experiments, it will be seen how the difficulties mentioned in the first paragraph of this article are overcome.

The gas concentration in the flask is practically unaltered by the actions of introducing the insects into, or removing them from, the animal chamber. Prior to the actual commencement of an experiment this chamber contains air when the rest of the apparatus is charged with a gas mixture and a certain quantity of fumigant. By flushing the animal chamber with three or four times its volume of gas mixture from the reserve flask, the air is substantially replaced by an atmosphere very similar in composition to that in the fumigation flask. As the whole operation described in section IV (8) occupies only about 1 min. the insects are submitted to almost the full concentration of fumigant instantly. At the termination of the experiment the composition of the mixture in the flask is preserved by turning the animal chamber through 90° before removing the insects.

The mixture in the flask is kept in motion by the intermittent action of the plunger, and a continuous flow is maintained through the animal chamber by the circulating pump.

A gas sample can be taken at any time during the experiment and the reduction in pressure in the flask compensated by a displacement of gas from the reserve flask. If the sample is taken a considerable time after the experiment has commenced an error is introduced by the fact that, owing to a slight loss of gas from the flask and connecting tubes, the mixture from the reserve flask contains a small excess of fumigant over that extracted in the sample. However, as the loss is small—about 0·5 per cent. per hour—and the size of the sample is only about 100 ml., the error introduced is negligible.

The apparatus is so constructed that gas mixtures of predetermined composition can be easily prepared. Other experiments which may be conducted by the use of this apparatus will be described in a later paper.

VII. Summary.

An account is given of a new type of laboratory apparatus for fumigation experiments with insects or other small animals, and the experimental procedure adopted is described. Reference is also made to appliances used by other investigators.

This work was suggested and supervised by Prof. J. W. Munro, to whom my thanks are due for advice and encouragement.

I also take this opportunity of thanking Dr A. B. P. Page for his help in devising the electrical equipment of the constant temperature cabinet and his useful criticism of the description here given.

The designing and construction of this apparatus and the experimental work carried out with it, which will be described in later papers, have been made possible by a grant made to the Entomology Department of the Imperial College of Science and Technology, by Imperial Chemical Industries, Ltd., to whom thanks are due for their generous financial help.

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EXPLANATION OF PLATE XXXVII.

Fig. 1. View of constant temperature cabinet from front and right side.

Fig. 2. View of cabinet from back and left side. *B*, felt-covered aperture; *C*, battery of cells for internal lighting; *D*, upper portion of cabinet; *DO*, door; *E*, support for *D*; *M*, manometer; *MO*, motor for working circulating pumps and plungers; *PU*, vacuum pump; R_1 and R_2, mercury reservoirs; *RE*, relay to thermostat; *RF*, reserve gas flask; *V*, pulley and shaft driven by *MO*.

(*Received March 7th*, 1934.)

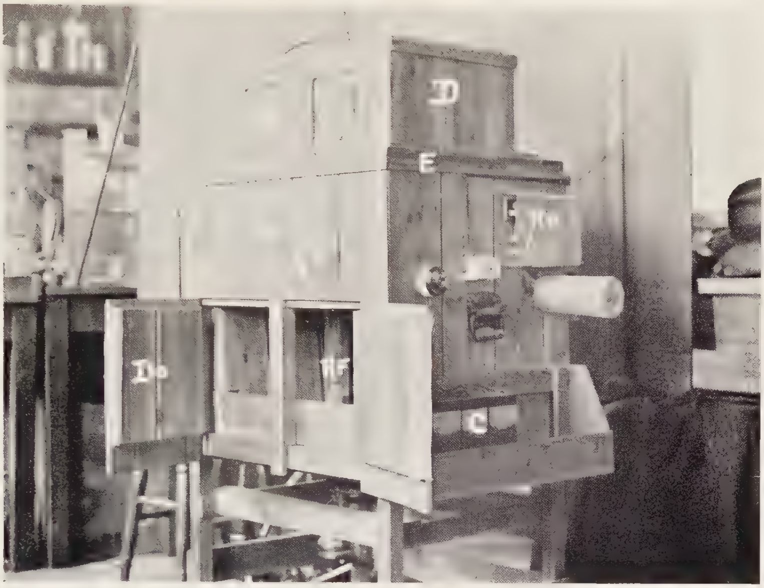

Fig. 1.

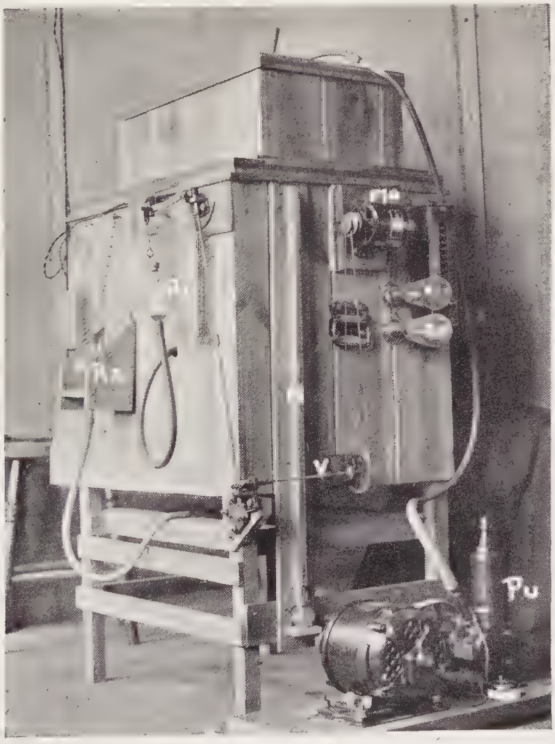

Fig. 2.

BOVINGDON.—An Improved Laboratory Apparatus for Fumigation Experiments (pp. 704–724).

REVIEWS

(1) *The Elements of Experimental Embryology.* By J. S. Huxley and G. R. De Beer. Pp. xii+514, text-figs. 221. Cambridge University Press. 1934. 25*s*.

(2) *Features in the Architecture of Physiological Function.* By J. Barcroft. Pp. x+368, text-figs. 106, tables XLIII. Cambridge University Press. 1934. 20*s*.

It is not easy for workers in applied biology to keep up-to-date with progress in the more rapidly advancing aspects of pure biology, and a good many applied biologists are dated by the viewpoints implicit in their work, these viewpoints being those current in pure science at the time when they entered the applied field. Yet if applied biology is to play its part in the advance of knowledge and the increased efficiency of practice it must keep in step with pure biology: an irregular lag period results in scientific unbalance, stultification of work and waste of time and mental energy. Of the aspects of biology in which swift progress is being made physiology is one of the more outstanding, and the new discoveries and new points of view which are accruing must have wide and deep influence on biological thought and eventually on biological application. For a considerable period physiological research was a relatively inchoate field, here extensively worked, there virgin territory, and it was not easy to see the underlying relationships in the huge masses of data gradually becoming available. Recent work, however, tends to co-ordinate this knowledge and to indicate the organic principles integrating its structure.

These newer researches, largely of a comparative nature, are often technically abstruse, and the literature is widely scattered, so that not only does the applied worker find them hard to come by and hard to read but he also finds it very difficult to synthesise them for himself or to fit them into his ideology. It is almost essential that the data be marshalled and their implications pointed out by broad-minded workers from within the actual fields of research, when the synthesis can be read with value by workers in other fields.

The two books here noted are of this order. Neither has directly applied significance, but both synthesise a great deal of modern research and are written from a comparative point of view by leading workers in the particular fields concerned, workers competent not only to evaluate the specific data but also to visualise the implications of these data in relation to the wider issues of biology. Neither book will help directly in the daily work of the applied biologist, but both books will help to keep the applied worker in step with pure biology in vital fields of research where the whole configuration of the biological landscape is being seen in new lights, giving a changed perspective and a new appreciation of the relative importance of the several features. By altering the facets and colouring of one's mental background and introducing novel ideas and fresh points of view books of this nature have a great importance outside their special field, and workers in applied biology will be well advised to note them. They are the first two volumes of a new "Cambridge Comparative Physiology" series edited by J. Barcroft and J. T. Saunders.

Barcroft's treatment of his subject is based upon a functional approach to physiology with the recognition of eight primary features in functional architecture: fixity of internal environment, storage of reserve environmental material (protein, carbohydrate, fat, salts and water), adaptive integration, "all-or-none" response, unit function, antagonism, maximal activity, and duplication of mechanism. The last chapter, "The chance that a phenomenon has a significance," in which the author approaches the metaphysical aspect of his work, is of quite unusual interest.

Huxley and De Beer have attempted "to give some account of the results of the experimental attack on the problem of the biology of differentiation—the production of an organised whole with differentiated parts out of an entirely or relatively undifferentiated portion of living material." The book is largely a discussion of the function of organisers in the gastrulation processes of Amphibia, with suggestion of the implications of research in this field and the extension of the concept to other stages of development and to other groups of organisms. Such epigenetic analysis of development leads directly to physiological genetics which, in turn, may lead to some comprehension of the basic processes involved in the action of particular Mendelian genes and so, ultimately, to a reorientation in applied genetics.

The books are well written and, coming from the Cambridge Press, are beautifully produced.

W. B. BRIERLEY.

The Agricultural Register, 1933–4. Oxford: Agricultural Economic Research Institute. 1934. Pp. vii+229. 3*s.* 6*d.* net.

In this country, during the last few years, the difficulties of agriculture have been serious and, about 1929, approached a condition to which the term crisis seems applicable. These difficulties have produced a change from a state of *laissez-faire*, through systems of subsidy or relief, to an immediate condition of active government intervention, which almost amounts to a revolution in the industry. The present condition is one of an attempt to reconstruct agriculture in some of its most important functions with a thorough revision of economic relations with other countries. For all who are directly interested in agriculture or rural life, or who are concerned in any way with the innumerable ramifications involved in the commercial exploitation of agricultural products, it is vital not only to keep in touch with these new schemes and alterations but to have a more or less detailed knowledge of them. Their varied nature, however, the rapidity with which they have been formulated and put into operation, and their numerous secondary effects, have made this extremely difficult even for those having easy access to reliable sources of information. Government blue books are always with us but are not always very intelligible, and the innumerable volumes on agriculture which appear are often behind the times and, by their polemical nature, frequently do more to obscure than clarify the issues. There was abundant need for an impartial agricultural register in which one could find a plain straightforward record of just exactly what schemes had been formulated with a precise account of the mechanism of their working. This need has been met by the present volume which is a record of the changes in agricultural legislation, organisation, supplies and prices during the year 1933–4. It simply states the facts and leaves one to draw whatever political, social and economic deductions one likes from them.

The first chapter is a wholly admirable statement in ten pages of the principles of the new agricultural policy. The second chapter contains in severely summarised but quite adequate form the acts of parliament, statutory rules and orders, and trade agreements of the past year. The third chapter, dealing with administrative action, contains an account of the reorganisation commissions on milk, pigs, fat stock, and eggs and poultry, the marketing schemes for hops, milk, pigs and bacon and potatoes, and the marketing boards for hops, milk and pigs and bacon. Chapter IV deals with prices and supplies and after a general introduction passes to a specific consideration of such commodities as meat, dairy products, eggs and poultry, crops, fruits and vegetables and wool and such requisites as fertilisers and feeding stuffs. Two short chapters then deal severally with the statistics of crops and grass and livestock, and with unemployment problems such as wages, labour and unemployment insurance. A final chapter of miscellanea contains an account of the weather during 1933, statements of the controversial subject of tithe rentcharge and agricultural credit, and a brief note on the committee on the slaughtering of livestock. The book concludes with an adequate index.

This volume is an extraordinarily useful compilation and the Oxford Agricultural Economics Research Institute is to be congratulated on its production. It is the intention to bring out further issues annually, and although, within its compass, it is a little difficult to see how it could be bettered, the Director of the Institute "will welcome suggestions from readers for extending its usefulness."

W. B. BRIERLEY.

A list of members of the Association of Applied Biologists was published in the *Annals of Applied Biology*, xx, No. 4 (Nov. 1933), pp. 778–87. The following additions and changes are to be noted. Will any member changing address please inform the Hon. Treasurer and one of the Hon. Secretaries.

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